Desai 09/819,098

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=> d que l1
L1             0 SEA FILE=REGISTRY AYVKVDSCPEEPQLIMKNNEEAEDYDDDLT|LICYKESVDQKGNQ
                 IMSDKRNVILFSVFDE|TFLTAQTLLMDLGQFLLSCHISSHQHDGME|TFLTAQTLLMDLGQF
                 LL[-F]CHISSHQHDGME/SQSP


=> d his 1

     (FILE 'MEDLINE, HCAPLUS, BIOSIS, EMBASE, SCISEARCH, AGRICOLA' ENTERED AT
     15:18:25 ON 22 OCT 2003)
L20           44 DUP REM L19 (40 DUPLICATES REMOVED)

=> d que l20
L2          4418 SEA KAUFMAN R?/AU
L3           134 SEA PIPE S?/AU
L4          3473 SEA AMANO K?/AU
L5          7898 SEA (L2 OR L3 OR L4)
L6           438 SEA L5 AND (FACTORVIII OR FACTOR(A) VIII OR FACTOR8 OR
                 FACTOR(A) 8)
L7            72 SEA L6 AND INACTIVAT?
L8            50 SEA L7 AND (ARG? OR ARGININE# OR PHE? OR PHENYLALANINE#)
L9         59282 SEA FACTORVIII OR FACTOR(A) VIII OR FACTOR8 OR FACTOR(A) 8
L10         5018 SEA L9 AND (ARG? OR ARGININE# OR PHE? OR PHENYLALANINE#)
L11         1750 SEA L10 AND (MUTAN? OR MUTAT? OR INSERT? OR DELET? OR SUBSTITUT
                 ? OR RESIDUE#)
L12          220 SEA L11 AND INACTIVAT?
L13          222 SEA L8 OR L12
L14           54 SEA L13 AND RESIST?
L15           12 SEA L13 AND 562
L16           30 SEA L13 AND 336
L17            7 SEA L13 AND 309
L18           27 SEA L13 AND (PHE309? OR ARG336? OR ARG562?)
L19           84 SEA (L14 OR L15 OR L16 OR L17 OR L18)
L20           44 DUP REM L19 (40 DUPLICATES REMOVED)


=> d ibib abs 120 1-44

L20  ANSWER 1 OF 44  HCAPLUS  COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER:         2003:610058  HCAPLUS
DOCUMENT NUMBER:          139:161308
TITLE:                    Inactivation resistant
                          procoagulant-active factor VIII
                          and therapeutic use for hemophilia
INVENTOR(S):              Kaufman, Randal J.; Pipe, Steven W.
PATENT ASSIGNEE(S):       University of Michigan, USA
SOURCE:                   U.S. Pat. Appl. Publ., 57 pp., Cont.-in-part of U.S.
                          Ser. No. 122,264.
                          CODEN: USXXCO
DOCUMENT TYPE:            Patent
LANGUAGE:                 English
FAMILY ACC. NUM. COUNT:   2
PATENT INFORMATION:

     PATENT NO.        KIND  DATE          APPLICATION NO.  DATE
     ---------------   ----  --------      ---------------  --------
     US 2003148953      A1   20030807      US 2002-283648   20021029
     WO 9740145         A1   19971030      WO 1997-US6563   19970424
         W:  AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE,
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Search completed by David Schreiber 308-4292

DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, US, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG

US 2002132306 A1 20020919 US 2001-819098 20010411

PRIORITY APPLN. INFO.:
US 1996-16117P P 19960424
US 1996-17785P P 19960515
WO 1997-US6563 A2 19970424
US 1997-980038 B1 19971126
US 2001-819098 A2 20010411
US 2002-122264 A2 20020411

AB The present invention provides novel purified and isolated nucleic acid sequences encoding procoagulant-active FVIII proteins. The nucleic acid sequences of the present invention encode amino acid sequences corresponding to known human FVIII sequences, wherein **residue Phe309** is **mutated**. The nucleic acid sequences of the present invention also encode amino acid sequences corresponding to known human FVIII sequences, wherein the APC cleavage sites, **Arg336** and Ile562, are **mutated**. The nucleic acid sequences of the present invention further encode amino acid sequences corresponding to known human FVIII sequences, wherein the B-domain is **deleted**, the von Willebrand factor binding site is **deleted**, a thrombin cleavage site is **mutated**, an amino acid sequence spacer is **inserted** between the A2- and A3-domains. Methods of producing the FVIII proteins of the invention, nucleotide sequences encoding such proteins, pharmaceutical compns. containing the nucleotide sequences or proteins, as well as methods of treating patients suffering from hemophilia, are also provided.

L20 ANSWER 2 OF 44 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2003219831 MEDLINE
DOCUMENT NUMBER: 22615925 PubMed ID: 12606556
TITLE: Mechanisms of factor Xa-catalyzed cleavage of the factor VIIIa A1 subunit resulting in cofactor **inactivation**.
AUTHOR: Nogami Keiji; Wakabayashi Hironao; Fay Philip J
CORPORATE SOURCE: Department of Biochemistry, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642, USA.
CONTRACT NUMBER: HL 30616 (NHLBI)
HL 38199 (NHLBI)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (2003 May 9) 278 (19) 16502-9.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200306
ENTRY DATE: Entered STN: 20030514
Last Updated on STN: 20030701
Entered Medline: 20030630

AB Activation of **factor VIII** by factor Xa is followed by proteolytic **inactivation** resulting from cleavage within the A1 subunit (**residues** 1-372) of factor VIIIa. Factor Xa attacks two sites in A1, **Arg(336)**, which precedes the highly acidic C-terminal region, and a recently identified site at Lys(36). By

using isolated A1 subunit as substrate for proteolysis, production of the terminal fragment, A1(37-**336**), was shown to proceed via two pathways identified by the intermediates A1(1-**336**) and A1(37-372) and generated by initial cleavage at **Arg**(**336**) and Lys(36), respectively. Appearance of the terminal product by the former pathway was 7-8-fold slower than the product obtained by the latter pathway. The isolated A1 subunit was cleaved slowly, independent of the presence of phospholipid. The A1/A3-C1-C2 dimer demonstrated an approximately 3-fold increased cleavage rate constant, and inclusion of phospholipid further enhanced this value by approximately 2-fold. Although association of A1 or A1(37-372) with A3-C1-C2 enhanced the rate of cleavage at **Arg**(**336**), inclusion of A3-C1-C2 did not affect the cleavage at Lys(36) in A1(1-**336**). A synthetic peptide 337-372 blocked the cleavage at Lys(36) (IC(50) = 230 microm) while showing little if any effect on cleavage at **Arg**(**336**). Proteolysis at Lys(36), and to a lesser extent **Arg**(**336**), was inhibited in a dose-dependent manner by heparin. These results suggest that **inactivating** cleavages catalyzed by factor Xa at Lys(36) and **Arg**(**336**) are regulated in part by the A3-C1-C2 subunit. Furthermore, cleavage at Lys(36) appears to be selectively modulated by the C-terminal acidic region of A1, a region that may interact with factor Xa via its heparin-binding exosite.

L20 ANSWER 3 OF 44 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 2003259171 EMBASE

TITLE: [Hemostatic parameters as hypercoagulability indicators in pregnancy].
PARAMETRI HEMOSTAZE KAO POKAZATELJI HIPERKOAGULABILNOSTI U TRUDNOCI.

AUTHOR: Vodnik T.; Ignjatovic S.; Majkic-Singh N.

CORPORATE SOURCE: T. Vodnik, Institut za Medicinsku Biohemiju, Klinicki Centar Srbije, Visegradska 26, 11000 Beograd, Yugoslavia

SOURCE: Jugoslovenska Medicinska Biokemija, (2003) 22/2 (119-126). Refs: 26
ISSN: 0354-3447 CODEN: JMBIEE

COUNTRY: Yugoslavia

DOCUMENT TYPE: Journal; Conference Article

FILE SEGMENT: 010 Obstetrics and Gynecology
025 Hematology
027 Biophysics, Bioengineering and Medical Instrumentation
029 Clinical Biochemistry

LANGUAGE: Serbian

SUMMARY LANGUAGE: English; Serbian

AB The concentrations of plasma proteins involved in the process of coagulation are changed during the normal pregnancy, interfering with balance between procoagulant and anticoagulant systems. These changes include the increased activity of coagulation factors, the increased fibrin production and suppression of fibrinolysis. In this way, the risk of blood loss is reduced by physiological mechanisms, but the risk of thrombosis becomes higher. Thrombosis of placental blood vessels gives rise to placental insufficiency, causing the repeated miscarriages, retardation of fetal growth, eclampsia, intrauterine death of fetus and pre-term delivery. The causes of these incidents are the changes occurring within protein C coagulation system. The central anticoagulant enzyme is APC, which **inactivates** the activated factors V and VIII by proteolysis and, in that manner, inhibits the production of thrombin. The **resistance** to APC is the impairment of blood coagulation

characterized by lower sensitivity to anticoagulant activity of APC. This disorder is in 95% of cases, caused by specific factor V gene point **mutation** and designated as F V Leiden. It is point **mutation** on locus 1691 where guanine is replaced by adenine what will lead to synthesis of modified F V molecule in which **arginine** is replaced by glutamine on locus 506. **Arginine** 506 is the locus of factor Xa binding, and it inhibits the **inactivation** of factor Va by the action of APC. The testing performed in pregnant women, with regular and complicated pregnancy, in different periods, has shown that fibrinogen, fibrin monomer, TAT complex and PAI were good markers of hipercoagulability in pregnancy. The values of protein C activity were within normal limits. Protein S values were below lower limits. Global activity of protein C-anticoagulant pathway and its relation with procoagulant system were presented by quantitative measurement using Pro C Global test, while determination of activated protein C by means of APC sensitivity test was used for identification of persons with APC **resistance**, e.g. with higher risk of thrombosis. Significantly lower PC-NR and APC-NR values were found in pregnant women with repeated miscarriages and in pregnant women with hypertension in relation to pregnant women with regular pregnancy. The results revealed significantly lower APC-anticoagulant activity in pregnancy, particularly in pregnancy associated with complications. Diagnostic accuracy of these parameters as markers of thrombotic changes in pregnancy was tested by ROC analysis. PC-NR and APC-NR showed satisfactory diagnostic accuracy as markers of thrombotic changes in pregnant women, more precisely, they were found to be good indicators of **resistance** to activated protein C in pregnancy.

L20 ANSWER 4 OF 44 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 2002:318288 SCISEARCH
THE GENUINE ARTICLE: 539AC
TITLE: Cofactor activities of factor VIIIa and A2 subunit following cleavage of A1 subunit at **Arg**(**336**)
AUTHOR: Rosenblum M E K; Schmidt K; Freas J; Mastri M; Fay P J (Reprint)
CORPORATE SOURCE: Univ Rochester, Med Ctr, Dept Biochem & Biophys, POB 610, 601 Elmwood Ave, Rochester, NY 14642 USA (Reprint); Univ Rochester, Sch Med & Dent, Dept Biochem & Biophys, Rochester, NY 14642 USA; Univ Rochester, Sch Med & Dent, Dept Med, Rochester, NY 14642 USA
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (5 APR 2002) Vol. 277, No. 14, pp. 11664-11669.
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3996 USA.
ISSN: 0021-9258.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 37

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Factor VIIIa consists of three subunits designated A1, A2, and A3-C1-C2. The isolated A2 subunit possesses limited cofactor activity in stimulating factor IXa-catalyzed activation of factor X. This activity is markedly enhanced by the A1 subunit (inter-subu-nit K-d = 1.8 mum). The C-terminal region of A1 subunit (**residues** 337-372) is thought to represent an A2-interactive site. This region appears critical to factor VIIIa, because proteolysis at **Arg**(**336**) by activated protein C or factor IXa is **inactivating**. A truncated A1 (A1(

336)) showed similar affinity for A2 subunit (Kd = 0.9 muM) and stimulated its cofactor activity to similar to50% that observed for native A1. However, A1336 was unable to reconstitute factor VIIIa activity in the presence of A2 and A3-C1-C2 subunits. Fluorescence anisotropy of fluorescein (F1)-FFR-factor IXa was differentially altered by factor VIIIa trimers containing either A1 or A1(**336**). Fluorescence energy transfer demonstrated that, although F1-A1(**336**)/A3-C1-C2 bound acrylodan-A2 with similar affinity as the native dimer, an increased inter-fluorophore separation was observed. These results indicate that the C-terminal region of A1 appears necessary to properly orient A2 subunit relative to factor IXa in the cofactor rather than directly stimulate A2 and elucidate the mechanism for cofactor **inactivation** following cleavage at this site.

L20 ANSWER 5 OF 44 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2002299851 MEDLINE
DOCUMENT NUMBER: 22005971 PubMed ID: 12010798
TITLE: Isolation and characterization of an antifactor V antibody causing activated protein C **resistance** from a patient with severe thrombotic manifestations.
AUTHOR: Kalafatis Michael; Simioni Paolo; Tormene Daniela; Beck Daniel O; Luni Sonia; Girolami Antonio
CORPORATE SOURCE: Department of Chemistry, Cleveland State University, The Cleveland Clinic Foundation, Cleveland, OH 44115, USA.. m.kalafatis@csuohio.du
CONTRACT NUMBER: HL34575 (NHLBI)
SOURCE: BLOOD, (2002 Jun 1) 99 (11) 3985-92. Journal code: 7603509. ISSN: 0006-4971.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200207
ENTRY DATE: Entered STN: 20020604
Last Updated on STN: 20020702
Entered Medline: 20020701

AB A 44-year-old woman with a history of severe thrombotic manifestations presented with a markedly reduced activated protein C-sensitivity ratio (APC-SR). DNA sequencing of and around the regions encoding the APC cleavage sites in the factor Va molecule excluded the presence of the factor VLeiden **mutation** and of other known genetic **mutations**. No antiphospholipid antibodies were present in the patient's plasma and both prothrombin time and activated partial thromboplastin time were normal. The total immunoglobulin fraction was isolated from the patient's plasma and found to induce severe APC **resistance** when added to normal plasma and to factor V-deficient plasma supplemented with increasing concentrations of factor V. Immunoblotting and immunoprecipitation experiments with the total immunoglobulin fraction purified from the patient's plasma demonstrated that the antibody recognizes factor V, is polyclonal, and has conformational epitopes on the entire factor V molecule (heavy and light chains, and B region). Thus, the immunoglobulin fraction interferes with the anticoagulant pathway involving factor V. The inhibitor was isolated by sequential affinity chromatography on protein G-Sepharose and factor V-Sepharose. The isolated immunoglobulin fraction inhibited factor Va **inactivation** by APC because of impaired cleavage at **Arg306** and **Arg506** of the heavy chain of the cofactor. The isolated immunoglobulin fraction was also found to inhibit the cofactor effect of factor V for the **inactivation** of **factor VIII**

by the APC/protein S complex. Our data provide for the first time the demonstration of an antifactor V antibody not related to the presence of antiphospholipid antibodies, which is responsible for thrombotic rather than hemorrhagic symptoms.

L20 ANSWER 6 OF 44 MEDLINE on STN
ACCESSION NUMBER: 2002216353 MEDLINE
DOCUMENT NUMBER: 21947498 PubMed ID: 11950687
TITLE: Factor V and thrombotic disease: description of a janus-faced protein.
AUTHOR: Nicolaes Gerry A F; Dahlback Bjorn
CORPORATE SOURCE: Department of Laboratory Medicine, Division of Clinical Chemistry, Lund University, The Wallenberg Laboratory, University Hospital Malmo, Malmo, Sweden.
SOURCE: ARTERIOSCLEROSIS, THROMBOSIS, AND VASCULAR BIOLOGY, (2002 Apr 1) 22 (4) 530-8. Ref: 107
Journal code: 9505803. ISSN: 1524-4636.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200205
ENTRY DATE: Entered STN: 20020416
Last Updated on STN: 20020507
Entered Medline: 20020506

AB The generation of thrombin by the prothrombinase complex constitutes an essential step in hemostasis, with thrombin being crucial for the amplification of blood coagulation, fibrin formation, and platelet activation. In the prothrombinase complex, the activated form of coagulation factor V (FVa) is an essential cofactor to the enzyme-activated factor X (FXa), FXa being virtually ineffective in the absence of its cofactor. Besides its procoagulant potential, intact factor V (FV) has an anticoagulant cofactor capacity functioning in synergy with protein S and activated protein C (APC) in APC-catalyzed **inactivation** of the activated form of **factor VIII**. The expression of anticoagulant cofactor function of FV is dependent on APC-mediated proteolysis of intact FV. Thus, FV has the potential to function in procoagulant and anticoagulant pathways, with its functional properties being modulated by proteolysis exerted by procoagulant and anticoagulant enzymes. The procoagulant enzymes factor Xa and thrombin are both able to activate circulating FV to FVa. The activity of FVa is, in turn, regulated by APC together with its cofactor protein S. In fact, the regulation of thrombin formation proceeds primarily through the upregulation and downregulation of FVa cofactor activity, and failure to control FVa activity may result in either bleeding or thrombotic complications. A prime example is APC **resistance**, which is the most common genetic risk factor for thrombosis. It is caused by a single point **mutation** in the FV gene (factor V(Leiden)) that not only renders FVa less susceptible to the proteolytic **inactivation** by APC but also impairs the anticoagulant properties of FV. This review gives a description of the dualistic character of FV and describes the gene-gene and gene-environment interactions that are important for the involvement of FV in the etiology of venous thromboembolism.

L20 ANSWER 7 OF 44 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 2002:411336 SCISEARCH

Search completed by David Schreiber 308-4292

THE GENUINE ARTICLE: 551EV
TITLE: The activated protein C (APC)-**resistant phenotype** of APC cleavage site **mutants** of recombinant factor V in a reconstituted plasma model
AUTHOR: Kolfschoten M V (Reprint); Dirven R J; Tans G; Rosing J; Vos H L; Bertina R M
CORPORATE SOURCE: Leiden Univ, Med Ctr, Ctr Hemostasis & Thrombosis Res, Dept Hematol, D2-19, POB 9600, NL-2300 RC Leiden, Netherlands (Reprint); Leiden Univ, Med Ctr, Ctr Hemostasis & Thrombosis Res, Dept Hematol, NL-2300 RC Leiden, Netherlands; UM, CARIM, Dept Biochem, Maastricht, Netherlands
COUNTRY OF AUTHOR: Netherlands
SOURCE: BLOOD COAGULATION & FIBRINOLYSIS, (APR 2002) Vol. 13, No. 3, pp. 207-215.
Publisher: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA.
ISSN: 0957-5235.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 43

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Recently, new missense **mutations** in the activated protein C (APC) cleavage sites of human factor V (FV) distinct from the R506Q (FV Leiden) **mutation** have been reported. These **mutations** affect the APC cleavage site at **arginine** (**Arg**) 306 in the heavy chain of activated FV. Whether these **mutations** result in APC **resistance** and are associated with a risk of thrombosis is not clear. The main objective of the present study was to identify the APC-**resistant phenotype** of FV molecules with different **mutations** in APC cleavage sites. To study this, recombinant FV **mutants** were reconstituted in FV-deficient plasma, after which normalized APC-sensitivity ratios (n-APC-SRs) were measured in activated partial thromboplastin time-based and Russell's Viper Venom time-based APC-**resistance** tests. The **mutations** introduced in FV were R306G, R306T, R506Q, R679A and combinations of these **mutations**. Based on the APC-sensitivity ratios, we conclude that the naturally occurring **mutations** at **Arg306** (i.e. FV HongKong and FV Cambridge) result in a mildly reduced sensitivity for APC (n-APC-SR, 0.74-0.87), whereas much lower values (n-APC-SR, 0.41-0.51) are obtained for the **mutation** at **Arg506** (FV Leiden). No effect on the n-APC-SR was observed for the recombinant FV **mutant** containing the single Ala679 **mutation**. Because reduced sensitivity for APC, not due to FV Leiden, is a risk factor for venous thrombosis, these data suggest that **mutations** at **Arg306** might be associated with a mild risk of venous thrombosis. (C) 2002 Lippincott Williams Wilkins.

ACCESSION NUMBER: 2002086857 EMBASE
TITLE: Proteolysis of protein C in pooled normal plasma and purified protein C by activated protein C (APC).
AUTHOR: Hassouna H.; Quinn C.
CORPORATE SOURCE: H. Hassouna, Department of Medicine, Michigan State University, B-239 Clinical Center, East Lansing, MI 48824-1313, United States. hassouna@msu.edu
SOURCE: Biophysical Chemistry, (19 Feb 2002) 95/2 (109-124).
Refs: 61

Search completed by David Schreiber 308-4292

ISSN: 0301-4622 CODEN: BICIAZ
PUBLISHER IDENT.: S 0301-4622(01)00267-8
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 025 Hematology
029 Clinical Biochemistry
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Protein C is a vitamin-K dependent zymogen of the anti-coagulant serine protease activated protein C (APC). In this paper, we report four lines of evidence that APC can activate protein C in pooled normal plasma, and purified protein C. First, the addition of APC to protein C-deficient plasma supplemented with protein C produces a prolongation of the clotting time of plasma that is proportional to the amount of protein C. This behavior was observed with APC from the Chromogenix APC **resistance** kit (Dia Pharm, Franklin, OH, USA) and from APC derived from the thrombin activation of human protein C (Enzyme Research Laboratories, South Bend, IN, USA). Secondly, using immunoblotting after gel electrophoresis, the disappearance of epitopes for monoclonal antibodies that recognize protein C but not APC indicates a time course for the activation by APC of protein C in pooled normal plasma and protein C purified from plasma. Thirdly, the same time course for the disappearance of protein C specific epitope can be followed using ELISA. Finally, protein C can be activated by APC as indicated by the increase in APC specific synthetic substrate Tryp-**Arg-Arg**-p nitroaniline hydrolysis. Kinetic data indicate a value of 4.7±0.4 mM(-1) s(-1) for the activation of protein C by APC under physiological conditions and in the presence of calcium. These observations document that APC must function not only in the **inactivation** of activated factors V and VIII, but also in the activation of protein C. This additional action of APC may be important to consider more broadly because of APC in the treatment of sepsis. Copyright .COPYRGT. 2002 Elsevier Science B.V.

L20 ANSWER 9 OF 44 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:338762 HCAPLUS
DOCUMENT NUMBER: 134:362292
TITLE: Methods of determining individual hypersensitivity to a pharmaceutical agent from gene expression profile
INVENTOR(S): Farr, Spencer
PATENT ASSIGNEE(S): Phase-1 Molecular Toxicology, USA
SOURCE: PCT Int. Appl., 222 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001032928	A2	20010510	WO 2000-US30474	20001103
WO 2001032928	A3	20020725		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

Search completed by David Schreiber 308-4292

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 1999-165398P P 19991105
US 2000-196571P P 20000411

AB The invention discloses methods, gene databases, gene arrays, protein arrays, and devices that may be used to determine the hypersensitivity of individuals to a given agent, such as drug or other chemical, in order to prevent toxic side effects. In one embodiment, methods of identifying hypersensitivity in a subject by obtaining a gene expression profile of multiple genes associated with hypersensitivity of the subject suspected to be hypersensitive, and identifying in the gene expression profile of the subject a pattern of gene expression of the genes associated with hypersensitivity are disclosed. The gene expression profile of the subject may be compared with the gene expression profile of a normal individual and a hypersensitive individual. The gene expression profile of the subject that is obtained may comprise a profile of levels of mRNA or cDNA. The gene expression profile may be obtained by using an array of nucleic acid probes for the plurality of genes associated with hypersensitivity. The expression of the genes predetd. to be associated with hypersensitivity is directly related to prevention or repair of toxic damage at the tissue, organ or system level. Gene databases arrays and apparatus useful for identifying hypersensitivity in a subject are also disclosed.

L20 ANSWER 10 OF 44 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2002:151708 BIOSIS
DOCUMENT NUMBER: PREV200200151708
TITLE: **Mutations** within the APC-binding regions of Factors V and VIII as potential risk factors for venous thromboembolism.
AUTHOR(S): Heit, John A. [Reprint author]; Allred, Carolyn C. [Reprint author]; Plumhoff, Elizabeth A. [Reprint author]; Roberts, Stacy C. [Reprint author]; Lokken, Troy G. [Reprint author]; Ye, Hong [Reprint author]; Fass, David N. [Reprint author]; Petterson, Tanya M. [Reprint author]; O'Fallon, William M. [Reprint author]; Melton, L. J. [Reprint author]
CORPORATE SOURCE: Hematology Research, Mayo Clinic and Foundation, Rochester, MN, USA
SOURCE: Blood, (November 16, 2001) Vol. 98, No. 11 Part 2, pp. 90b. print.
Meeting Info.: 43rd Annual Meeting of the American Society of Hematology, Part 2. Orlando, Florida, USA. December 07-11, 2001. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 21 Feb 2002
Last Updated on STN: 26 Feb 2002

AB We hypothesized that **mutations** within the respective activated protein C (APC)-binding regions on the heavy chains of Factor (f)V (fV amino acids 1865-1874) or fVIII (fVIII amino acids 2009-2018) could alter APC binding affinity, produce an APC-**resistance phenotype**, and cause venous thromboembolism (VTE). To test this hypothesis, we used denaturing high pressure liquid chromatography to screen the fV and fVIII gene sequence encoding for amino acid **residues** fV1839-1874 and fVIII1981-2019 for unknown sequence variations using genomic DNA from two random samples (n=300) of

consecutive, objectively confirmed VTE patients referred to the Mayo Clinic Special Coagulation Laboratory for a clinical suspicion of thrombophilia, and from 14 VTE patients with APC-**resistance** who were non-carriers for the fV R506Q (Leiden) **mutation** and had no other cause for acquired APC-**resistance**. No **mutations** within these respective fV and fVIII gene sequences were discovered. However, a chronically-anticoagulated male patient with recurrent VTE was hemizygous for a novel fVIII6028T(R) C nucleotide change, encoding for a serine1991 to proline (fVIII S1991P) **substitution**. The patient also was a heterozygous carrier for the fV R506Q **mutation**. In an aPTT-based plasma fVIII activity assay, fVIII S1991P showed normal sensitivity to **inactivation** by exogenous APC. In an aPTT-based "fV activity" assay using fVIII-deficient substrate that also was immunodepleted of fV, the patient's plasma showed a sensitivity to exogenous APC **inactivation** that was similar to heterozygous fV R506Q plasma and intermediate between homozygous fV R506Q and normal plasma. Conclusions: 1) **Mutations** within the fV and fVIII gene sequence encoding for amino acid **residues** fV2009-2018 and fVIII1865-1874 are not common risk factors for VTE. 2) Based on the normal sensitivity of fVIII S1991P to APC **inactivation**, fVIII S1991 does not appear to be important for APC binding although this requires confirmation in direct binding assays.

L20 ANSWER 11 OF 44 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2002:129593 BIOSIS
DOCUMENT NUMBER: PREV200200129593
TITLE: Cofactor activity associated with the isolated A2 subunit of factor VIIIa is stimulated by a truncated A1 subunit lacking C-terminal **residues** 337-372.
AUTHOR(S): Rosenblum, Mary Koszelak [Reprint author]; Freas, Jan [Reprint author]; Mastri, Maria [Reprint author]; Fay, Philip J. [Reprint author]
CORPORATE SOURCE: Biochemistry and Biophysics, University of Rochester, Rochester, NY, USA
SOURCE: Blood, (November 16, 2001) Vol. 98, No. 11 Part 1, pp. 38a. print.
Meeting Info.: 43rd Annual Meeting of the American Society of Hematology, Part 1. Orlando, Florida, USA. December 07-11, 2001. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
Conference; (Meeting Poster)
LANGUAGE: English
ENTRY DATE: Entered STN: 6 Feb 2002
Last Updated on STN: 26 Feb 2002

AB Factor VIIIa, the cofactor for factor IXa, is comprised of three subunits designated A1, A2 and A3-C1-C2. Reconstitution of the isolated subunits generates factor VIIIa cofactor activity. Interestingly, the isolated A2 subunit possesses limited cofactor activity in stimulating factor IXa-catalyzed activation of factor X. However, this effect is enhanced greater than 10-fold in the presence of saturating levels of A1 subunit, which likely binds to and influences the conformation of A2 subunit rather than directly interacting with other reaction components. The C-terminal region of A1 subunit (**residues** 337-372) represents an A2-interactive site and is implicated in A2 subunit retention in the factor VIIIa trimer following cleavage of the **factor VIII** heavy chain during cofactor activation. This region appears critical to factor VIIIa function since proteolysis at **Arg337** by

activated protein C (APC) contributes to cofactor **inactivation.** However, a peptide corresponding to this sequence failed to stimulate the A2-dependent cofactor activity. To determine whether **residues** other than this C-terminal region of A1 were important for its functional interaction with A2, the A1 subunit was truncated with bovine APC following cleavage at **Arg337.** The resultant product, designated A1-**336** was purified following gel filtration to remove the **336**-372 fragment and evaluated for its capacity to stimulate isolated A2 subunit, as well as reconstitute factor VIIIa activity, using a factor Xa generation assay employing purified components. Results showed that the truncated A1 (800 nM) stimulated the activity of isolated A2 subunit (400 nM) apprx5-fold compared with a near 20-fold stimulation using an equivalent concentration of native A1 subunit. Addition of the 337-372 peptide (up to 40 muM) to A1-**336** did not increase its level of A2-stimulating activity. The fluorescence anisotropy of fluorescein-**Phe-Phe-Arg** labeled-factor IXa in the presence of A2 subunit was similarly increased by either native or the truncated A1 subunit. Alternatively, the truncated A1 failed to reconstitute factor VIIIa activity in the presence of A2 and A3-C1-C2 subunits. These results indicate that sequences(s) distinct from the C-terminal region A1 subunit interact with A2 and contribute to the cofactor effect of factor VIIIa. Furthermore, this region of A1 subunit appears necessary to orient, as well as retain A2 subunit in the factor VIIIa trimer.

L20 ANSWER 12 OF 44 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 2000:388063 SCISEARCH
THE GENUINE ARTICLE: 314VF
TITLE: Factor V antigen levels and venous thrombosis - Risk profile, interaction with factor V Leiden, and relation with **factor VIII** antigen levels
AUTHOR: Kamphuisen P W; Rosendaal F R; Eikenboom J C J; Bos R; Bertina R M (Reprint)
CORPORATE SOURCE: LEIDEN UNIV, MED CTR, CTR HEMOSTASIS & THROMBOSIS RES, C2-R, POB 9600, NL-2300 RC LEIDEN, NETHERLANDS (Reprint); LEIDEN UNIV, MED CTR, CTR HEMOSTASIS & THROMBOSIS RES, NL-2300 RC LEIDEN, NETHERLANDS; LEIDEN UNIV, MED CTR, DEPT CLIN EPIDEMIOL, NL-2300 RC LEIDEN, NETHERLANDS; TNO PREVENT & HLTH, GAUBIUS LAB, LEIDEN, NETHERLANDS
COUNTRY OF AUTHOR: NETHERLANDS
SOURCE: ARTERIOSCLEROSIS THROMBOSIS AND VASCULAR BIOLOGY, (MAY 2000) Vol. 20, No. 5, pp. 1382-1386.
Publisher: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA 19106-3621.
ISSN: 1079-5642.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 35

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Clotting factor V has a dual function in coagulation: after activation, procoagulant factor V stimulates the formation of thrombin, whereas anticoagulant factor V acts as a cofactor for activated protein C (APC) in the degradation of **factor VIII**/VIIIa, thereby reducing thrombin formation. In the present study, we evaluated whether plasma factor V levels, either decreased or increased, are associated with venous thrombosis. High procoagulant factor V levels may enhance prothrombinase activity and increase the thrombosis risk. Low anticoagulant factor V levels could reduce APC-cofactor activity in the **factor**

VIII inactivation (APC-**resistant phenotype**), which might also promote thrombosis. Low factor V levels in combination with factor V Leiden could lead to a more severe APC-**resistant phenotype** (pseudohomozygous APC **resistance**). To address these issues, we have measured factor V antigen (factor V:AE) levels in 474 patients with thrombosis and 474 control subjects that were part of the Leiden Thrombophilia Study (LETS). Factor V:Ag levels increased by 7.6 U/dL for every successive 10 years of age. Mean factor V:AE levels were 134 (range 41 to 305) U/dL in patients and 132 (range 47 to 302) U/dL in controls. Neither high nor low factor V:Ag levels were associated with venous thrombosis. We found that factor V:Ag and **factor VIII** antigen levels in plasma were correlated, but factor V did not modify the thrombotic risk of high **factor VIII** levels. The normalized APC ratio was not influenced by the factor V:Ag level in subjects with or without factor V Leiden. We conclude that neither low nor high factor V:Ag levels are associated with venous thrombosis and that factor V:Ag levels do not mediate the thrombotic risk associated with high **factor VIII** levels.

L20 ANSWER 13 OF 44 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1999:245753 HCAPLUS
DOCUMENT NUMBER: 131:30148
TITLE: Cleavage of factor V at **arg** 506 by activated protein C and the expression of anticoagulant activity of factor V
AUTHOR(S): Thorelli, Elisabeth; **Kaufman, Randal J.**; Dahlback, Bjorn
CORPORATE SOURCE: Department of Clinical Chemistry, University Hospital Malmo, Lund University, Malmo, S-205 02, Swed.
SOURCE: Blood (1999), 93(8), 2552-2558
CODEN: BLOOAW; ISSN: 0006-4971
PUBLISHER: W. B. Saunders Co.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Activated protein C (APC) inhibits coagulation by cleaving and **inactivating** procoagulant factor Va (FVa) and factor VIIIa (FVIIIa). FV, in addition to being the precursor of FVa, has anticoagulant properties; functioning in synergy with protein S as a cofactor of APC in the inhibition of the FVIIIa-factor IXa (FIXa) complex. FV:Q506 isolated from an individual homozygous for APC-**resistance** is less efficient as an APC-cofactor than normal FV (FV·R506). To investigate the importance of the three APC cleavage sites in FV (**Arg**-306, **Arg**-506, and **Arg**-679) for expression of its APC-cofactor activity, four recombinant FV **mutants** (FV:Q306, FV:Q306/Q506, FV:Q506, and FV:Q679) were tested. FV **mutants** with Gln (Q) at position 506 instead of **Arg** (R) were found to be poor APC-cofactors, whereas **Arg** to Gln **mutations** at positions 306 or 679 had no neg. effect on the APC-cofactor activity of FV. The loss of APC-cofactor activity as a result of the **Arg** -506 to Gln **mutation** suggested that APC-cleavage at **Arg** -506 in FV is important for the ability of FV to function as an APC-cofactor. Using Western blotting, it was shown that both wild-type FV and **mutant** FV was cleaved by APC during the FVIIIa inhibition. At optimum concns. of wild-type FV (11 nmol/L) and protein S (100 nmol/L), FVIIIa was found to be highly sensitive to APC with maximum inhibition occurring at less than 1 nmol/L APC. FV:Q506 was inactive as an APC-cofactor at APC-concns. ≤ 1 nmol/L and only partially active at higher APC concns. Our results show that increased expression of FV

anticoagulant activity correlates with APC-mediated cleavage at **Arg**-506 in FV, but not with cleavage at **Arg**-306 nor at **Arg**-679.

REFERENCE COUNT: 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L20 ANSWER 14 OF 44 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 1999:850478 SCISEARCH
THE GENUINE ARTICLE: 251JP
TITLE: Influence of **factor VIII**/von Willebrand complex on the activated protein C-**resistance phenotype** and on the risk for venous thromboembolism in heterozygous carriers of the factor V Leiden **mutation**
AUTHOR: DeMitrio V (Reprint); Marino R; Scaraggi F A; DiBari L; Giannoccaro F; Petronelli M; Ranieri P; Tannoia N; Schiraldi O
CORPORATE SOURCE: VIA TANZI 43, I-70121 BARI, ITALY (Reprint); UNIV BARI, SCH MED, CTR EMOSTASI & TROMBOSI, I-70124 BARI, ITALY; UNIV BARI, SCH MED, DEPT MED INTERNA, CATTEDRA EMATOL 2, I-70124 BARI, ITALY
COUNTRY OF AUTHOR: ITALY
SOURCE: BLOOD COAGULATION & FIBRINOLYSIS, (OCT 1999) Vol. 10, No. 7, pp. 409-416.
Publisher: LIPPINCOTT WILLIAMS & WILKINS, 227 EAST WASHINGTON SQ, PHILADELPHIA, PA 19106.
ISSN: 0957-5235.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 26

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB High factor WI plasma levels have been shown to represent a common increased risk for venous thromboembolism (VTE) and may cause an activated protein C (APC) **resistance** in the absence of the factor V Leiden **mutation**, but there are no studies specifically aimed to establish if high **factor VIII** and von Willebrand factor (vWF) concentrations may influence the APC sensitivity ratio (APC-SR) and increase the risk for VTE in the presence of the factor V Leiden **mutation**. For this purpose, we performed a retrospective case-control study to investigate the influence of the procoagulant **factor VIII** (WI:C) and the antigen of vWF (vWF:Ag) on the normalized APC-SR (n-APC-SR) and on the risk for VTE, in two selected groups of 30 symptomatic (Group I) and 32 asymptomatic (Group II) related heterozygotes for the factor V Leiden **mutation**. Differences between the two groups (Group I versus Group II) were: n-APC-SR, 0.57 +/- 0.06 versus 0.63 +/- 0.08, P = 0.001; **factor VIII**:C, 1.49 +/- 0.42 versus 1.13 +/- 0.28 IU/ml, P < 0.001; vWF:Ag, 1.46 +/- 0.53 versus 1.26 +/- 0.32 IU/ml, NS. As a whole (Group I + Group II), Pearson correlation coefficients were: n-APC-SR versus **factor VIII**:C, r = -0.410, P = 0.001; n-APC-SR versus vWF:Ag, r = -0.**309**, P = 0.01; **factor VIII**:C versus vWF:Ag, r = +0.640, P < 0.0001. The relative risk for VTE in individuals with the **factor VIII**:C concentration >1.5 IU/ml was 2.5 (95% confidence interval 1.6-3.9). We concluded that high **factor VIII**:C levels, probably in the effect of vWF, play a determinant role in worsening the APC-**resistance phenotype** and represent a common additional risk factor for VTE in heterozygous carriers of the factor V Leiden **mutation**. (C) 1999 Lippincott Williams &

Wilkins.

L20 ANSWER 15 OF 44 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 1999371559 MEDLINE
DOCUMENT NUMBER: 99371559 PubMed ID: 10443959
TITLE: Activated protein C **resistance** and thrombosis: molecular mechanisms of hypercoagulable state due to FVR506Q **mutation**.
AUTHOR: Dahlback B
CORPORATE SOURCE: Department of Clinical Chemistry, University of Lund, University Hospital, Malmo, Sweden.. bjorn.dahlback@klkemi.mas.lu.se
SOURCE: SEMINARS IN THROMBOSIS AND HEMOSTASIS, (1999) 25 (3) 273-89. Ref: 188
Journal code: 0431155. ISSN: 0094-6176.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199912
ENTRY DATE: Entered STN: 20000113
Last Updated on STN: 20000113
Entered Medline: 19991210

AB Inherited **resistance** to activated protein C (APC) is the most common genetic risk factor of venous thrombosis. It is caused by a single point **mutation** in the factor (F)V gene which predicts replacement of **Arg506** with a Gln (FVR506Q, FV: Q506 or FV Leiden). This **mutation** affects the function of the protein C system, a physiologically important natural anticoagulant pathway. APC inhibits coagulation by cleaving a limited number of peptide bonds in both intact and activated forms of factor V (FV/FVa) and **factor VIII** (FVIII/FVIIIa). Degradation of FVa by APC is stimulated by protein S, whereas **inactivation** of FVIIIa requires the synergistic cofactor function of protein S and FV proteolytically modified by APC. Thus, FV has the potential to express opposing functions, as a procoagulant after cleavage by thrombin or FXa and as an anticoagulant after cleavage by APC. The FVR506Q **mutation** not only confers partial **resistance** of FVa to APC but also impairs the degradation of FVIIIa because APC-mediated cleavage of FV at **Arg506** is required for expression of the anticoagulant activity of FV. The impaired degradation of both FVIIIa and FVa yield a hypercoagulable state conferring a lifelong increased risk of thrombosis. The FV **mutation** is common in Caucasians, whereas it is rarely found among other groups worldwide. In patients with severe thrombophilia having other inherited defects such as deficiencies of protein S, protein C, or antithrombin, APC **resistance** is often found as a contributing genetic risk factor. Individuals with combined genetic defects have a high risk of thrombosis, and it is now generally accepted that thrombophilia is a multigenetic disease.

L20 ANSWER 16 OF 44 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 1999338498 MEDLINE
DOCUMENT NUMBER: 99338498 PubMed ID: 10410309
TITLE: Synthesis of recombinant blood coagulation **factor VIII** (FVIII) heavy and light chains and reconstitution of active form of FVIII.
AUTHOR: Oh S H; Lee M Y; Song D W

Search completed by David Schreiber 308-4292

CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, College of Medicine, Yonsei University, Seoul, Korea..
shoh@yumc.yonsei.ac.kr
SOURCE: EXPERIMENTAL AND MOLECULAR MEDICINE, (1999 Jun 30) 31 (2) 95-100.
Journal code: 9607880. ISSN: 1226-3613.
PUB. COUNTRY: KOREA (SOUTH)
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199909
ENTRY DATE: Entered STN: 19991005
Last Updated on STN: 19991005
Entered Medline: 19990917

AB FVIII is synthesized as a single chain precursor of approximately 280 kD with the domain structure of A1-A2-B-A3-C1-C2 and it circulates as a series of metal ion-linked heterodimers that result from cleavages at B-A3 junction as well as additional cleavages within B domain. **Factor VIII** is converted to its active form, factor VIIIa, upon proteolytic cleavages by thrombin and is a heterotrimer composed of the A1, A2, and A3-C1-C2 subunits. A1 subunits of factor VIIIa terminates with 36 **residue** segment (Met337-**Arg372**) rich in acidic **residues**. This segment is removed after cleavages at **Arg336** by activated protein C, which results in **inactivation** of the cofactor. In the present study, site-directed mutagenesis of FVIII at **Arg336** to Gln336 was performed in order to produce an **inactivation resistant mutant** rFVIII (rFVIIIm) with an extended physiological stability. A recombinant **mutant** heavy chain of FVIII (rFVIII-Hm; **Arg336** to Gln336) and wild-type light chain of FVIII (rFVIII-L) were expressed in Baculovirus-insect cell (Sf9) system, and a biologically active recombinant **mutant** FVIII (rFVIIIm) was reconstituted from rFVIII-Hm and rFVIII-L in the FVIII-depleted human plasma containing 40 mM CaCl2. The rFVIIIm exhibited cofactor activity of FVIIIa (2.85 x 10(-2) units/mg protein) that sustained the high level activity during in vitro incubation at 37 degrees C for 24 h, while the cofactor activity of normal plasma was declined steadily for the period. These results indicate that rFVIIIm (**Arg336** to Gln336) expressed in Baculovirus-insect cell system is **inactivation resistant** in the plasma coagulation milieu and may be useful for the treatment of hemophilia A.

L20 ANSWER 17 OF 44 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 1999:209184 SCISEARCH
THE GENUINE ARTICLE: 174JY
TITLE: Mechanisms that regulate the anticoagulant function of coagulation factor V
AUTHOR: Thorelli E (Reprint)
CORPORATE SOURCE: LUND UNIV, MALMO UNIV HOSP, DEPT CLIN CHEM, S-20502 LUND, SWEDEN (Reprint)
COUNTRY OF AUTHOR: SWEDEN
SOURCE: SCANDINAVIAN JOURNAL OF CLINICAL & LABORATORY INVESTIGATION, (FEB 1999) Vol. 59, Supp. [229], pp. 19-26.
Publisher: SCANDINAVIAN UNIVERSITY PRESS, PO BOX 2959 TOYEN, JOURNAL DIVISION CUSTOMER SERVICE, N-0608 OSLO, NORWAY.
ISSN: 0036-5513.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; CLIN
LANGUAGE: English

REFERENCE COUNT: 35

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Coagulation factor V is composed of domains A1-A2-B-A3-C1-C2 and is activated by thrombin through proteolytic cleavage at **Arg** 709, **Arg** 1018 and **Arg** 1545. Upon thrombin activation, the B-domain is released and the active factor Va is formed by the heavy (A1-A2) and light chains (A3-C1-C2). Factor Va functions as an essential cofactor to factor Xa in the conversion of prothrombin to thrombin during coagulation. Recently it was shown that coagulation factor V, apart from being a precursor form to the procoagulant factor Va, also has anticoagulant properties, as it functions as a cofactor to activated protein C (APC). APC is a member of the anticoagulant pathway and downregulates the coagulation process through proteolytic **inactivation** of factors VIII/VIIIa and factors V/Va. In a factor VIIIa degradation assay, the APC-mediated **inactivation** of factor VIIIa is potentiated by the synergistic cofactors protein S and factor V. Protein S alone has little cofactor activity, whereas in the presence of factor V it is dramatically enhanced. This study provides insights into the molecular mechanisms that regulate the anticoagulant activity of factor V. Thrombin cleavage of factor V occurs in a sequential order. The thrombin cleavage site **Arg** 1545 is kinetically less favored than the other two sites, and cleavage at this site is the last to occur during thrombin activation of factor V. As a consequence of this, different activation intermediates exist that express different levels of procoagulant activity. The anticoagulant activities of these intermediates have now been studied. It was found that factor V could be cleaved by thrombin at both **Arg** 709 and **Arg** 1018 and still work fully as a cofactor to APC, whereas cleavage at **Arg** 1545 completely abolished the anticoagulant activity of factor V. This suggests that the APC cofactor function of factor V depends on the B-domain remaining attached to the A3 domain. This study further shows that APC converts coagulation factor V into a member of the anticoagulant pathway by cleaving factor V in the A2 domain at **Arg** 506. By cleavage of factor V, APC not only produces an anticoagulant cofactor, but at the same time eliminates the pool of procoagulant factor V, since APC cleaved factor V will have no future asa cofactor in the coagulation. The unique way by which APC and thrombin, through proteolytic cleavage, can convert factor V into either an anticoagulant or a procoagulant adds to the intriguing mechanisms that balance the procoagulant and anticoagulant forces.

L20 ANSWER 18 OF 44 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN

ACCESSION NUMBER: 1998:457285 SCISEARCH

THE GENUINE ARTICLE: ZT469

TITLE: The structure and function of murine factor V and its **inactivation** by protein C

AUTHOR: Yang T L; Cui J S; Rehumtulla A; Yang A; Moussalli M; **Kaufman R J**; Ginsburg D (Reprint)

CORPORATE SOURCE: UNIV MICHIGAN, SCH MED, DEPT HUMAN GENET, 4520 MSRB I, 1150 W MED CTR DR, ANN ARBOR, MI 48109 (Reprint); UNIV MICHIGAN, SCH MED, DEPT HUMAN GENET, ANN ARBOR, MI 48109; UNIV MICHIGAN, SCH MED, DEPT RADIAT ONCOL, ANN ARBOR, MI 48109; UNIV MICHIGAN, SCH MED, DEPT BIOL CHEM, ANN ARBOR, MI 48109; UNIV MICHIGAN, SCH MED, DEPT INTERNAL MED, ANN ARBOR, MI 48109; UNIV MICHIGAN, HOWARD HUGHES MED INST, ANN ARBOR, MI 48109

COUNTRY OF AUTHOR: USA

SOURCE: BLOOD, (15 JUN 1998) Vol. 91, No. 12, pp. 4593-4599. Publisher: W B SAUNDERS CO, INDEPENDENCE SQUARE WEST

Search completed by David Schreiber 308-4292

CURTIS CENTER, STE 300, PHILADELPHIA, PA 19106-3399.
ISSN: 0006-4971.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; CLIN
LANGUAGE: English
REFERENCE COUNT: 61

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Factor V (FV) is a central regulator of hemostasis, serving both as a critical cofactor for the prothrombinase activity of factor Xa and the target for proteolytic **inactivation** by the anticoagulant, activated protein C (APC). To examine the evolutionary conservation of FV procoagulant activity and functional **inactivation** by APC, we cloned and sequenced the coding region of murine FV cDNA and generated recombinant wild-type and **mutant** murine FV proteins. The murine FV cDNA encodes a 2,183-amino acid protein. Sequence comparison shows that the A1-A3 and C1-C2 domains of FV are highly conserved, demonstrating greater than 84% sequence identity between murine and human, and 60% overall amino acid identity among human, bovine, and murine FV sequences. In contrast, only 35% identity among all three species is observed for the poorly conserved B domain. The **arginines** at all thrombin cleavage sites and the R305 and R504 APC cleavage sites (corresponding to amino acid **residues** R306 and R506 in human FV) are invariant in all three species. Point **mutants** were generated to **substitute** glutamine at R305; R504, or both (R305/R504). Wild-type and all three **mutant** FV recombinant proteins show equivalent FV procoagulant activity. Single **mutations** at R305 or R504 result in partial **resistance** of FV to APC **inactivation**, whereas recombinant murine RI carrying both **mutations** (R305Q/R504Q) is nearly completely APC **resistant**. Thus, the structure and function of FV and its interaction with APC are highly conserved across mammalian species. (C) 1998 by The American Society of Hematology.

L20 ANSWER 19 OF 44 MEDLINE on STN DUPLICATE 5

ACCESSION NUMBER: 1998189525 MEDLINE
DOCUMENT NUMBER: 98189525 PubMed ID: 9531040

TITLE: **Mutation** at either **Arg336** or **Arg562** in **factor VIII** is insufficient for complete **resistance** to activated protein C (APC)-mediated **inactivation**: implications for the APC **resistance** test.

AUTHOR: **Amano K**; Michnick D A; Moussalli M; **Kaufman R J**
CORPORATE SOURCE: Howard Hughes Medical Institute, Department of Biological Chemistry, University of Michigan Medical Center, Ann Arbor 48109-0650, USA.

CONTRACT NUMBER: R01 HL52173 (NHLBI)
R01 HL53777 (NHLBI)
SOURCE: THROMBOSIS AND HAEMOSTASIS, (1998 Mar) 79 (3) 557-63.
Journal code: 7608063. ISSN: 0340-6245.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199805
ENTRY DATE: Entered STN: 19980529
Last Updated on STN: 19990129
Entered Medline: 19980521

AB Activated protein C (APC)-mediated **inactivation** of

factor VIII (FVIII) correlates with cleavage at either **Arg336** and/or **Arg562**. To elucidate the APC cleavage requirements for **inactivation** of FVIII, APC cleavage site **mutants** in FVIII (R336I, R562K and R336I/R562K) were made by site-directed mutagenesis. Analysis of these FVIII **mutants** expressed in COS-1 monkey cells demonstrated the thrombin-cleaved **mutant** R562K was **resistant** to APC cleavage at **residue 562** but not at **Arg336** and the thrombin cleaved **mutant** R336I was mostly **resistant** to APC cleavage at **residue 336**, but was sensitive to APC cleavage at **Arg562**. The double **mutant** R336I/R562K was mostly **resistant** to cleavage at **residue 336** and completely **resistant** to cleavage at **residue 562**. Thus, APC cleavage of FVIII does not require a specific order of cleavage at either **residue**. The functional **inactivation** by APC was studied using partially purified preparations of FVIII expressed in Chinese hamster ovary cells. Both single **mutants** were **inactivated** at similar rates but slower than wild-type FVIII, whereas the double **mutant** R336I/R562K was **resistant** to **inactivation**. The ability of a commercially available APC-**resistance** assay kit to detect APC **resistant** FVIII was tested by reconstituting FVIII deficient plasma with the APC **resistant mutants**. Only the R336I/R562K demonstrated a reduced APC-**resistance** ratio, indicating that this assay can not detect the single APC cleavage site **mutant** of FVIII. These results suggest that APC-mediated cleavage at either **Arg336** or **Arg562** partially **inactivate** FVIII.

L20 ANSWER 20 OF 44 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 1998:708951 SCISEARCH
THE GENUINE ARTICLE: 118RP
TITLE: Interaction of the A1 subunit of factor VIIIa and the serine protease domain of Factor X identified by zero-length cross-linking
AUTHOR: Lapan K A; Fay P J (Reprint)
CORPORATE SOURCE: UNIV ROCHESTER, MED CTR, VASC MED UNIT, SCH MED & DENT, DEPT BIOCHEM & BIOPHYS, POB 610, ROCHESTER, NY 14642 (Reprint); UNIV ROCHESTER, MED CTR, VASC MED UNIT, SCH MED & DENT, DEPT BIOCHEM & BIOPHYS, ROCHESTER, NY 14642; UNIV ROCHESTER, SCH MED & DENT, DEPT MED, ROCHESTER, NY 14642
COUNTRY OF AUTHOR: USA
SOURCE: THROMBOSIS AND HAEMOSTASIS, (SEP 1998) Vol. 80, No. 3, pp. 418-422.
Publisher: F K SCHATTAUER VERLAG GMBH, P O BOX 10 45 45, LENZHALDE 3, D-70040 STUTTGART, GERMANY.
ISSN: 0340-6245.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 40

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We have previously used a solid phase binding assay to localize a Factor X (FX) interactive site to the acidic C-terminus of the A1 subunit of FVIIIa (Lapan KA. Fay PJ. J Biol Chem 1997; 272: 2082-2088). The complex of FVIII-FX was made covalent following reaction with the zero-length cross-linking reagent 1-ethyl-3-(3-dimethylaminopropyl-)carbodiimide hydrochloride (EDC). Western blotting of the thrombin-cleaved complex showed that the A1 subunit of FVIIIa associated

with FX heavy chain. The FX-AI product was also detected following cross-linking to the A1/A3-C1-C2 dimer, but not the activated protein C-cleaved A1(**336**)/A3-C1-C2 form, indicating that a **residue**(s) in the region spanning Met(337)-**Arg**(372) contributed to the intermolecular ion pair(s). A synthetic peptide to this acidic region (FVIII337-372) cross-linked to FX and the product was alkaline **resistant** indicating that amide linkage(s) were formed. Sequence analysis of the FX-FVIII337-372 adduct suggested that the first 12 NH2-terminal **residues** of the FX and peptide do not participate in cross-link formation. Conversion of the cross-linked product to FXa by RW-X showed that the peptide was associated with the serine protease-forming domain of the heavy chain. These results indicate that the association of FVIIIa and FX occurs from a salt linkage(s) formed between **residues** of the Al acidic C-terminus of the cofactor (within **residues** 349-372) and the serine protease-forming domain of the substrate.

L20 ANSWER 21 OF 44 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1997:718000 HCAPLUS
DOCUMENT NUMBER: 127:356538
TITLE: construction of **inactivation resistant factor VIII** procoagulant and applications to hemophilia treatment
INVENTOR(S): **Kaufman, Randal J.; Pipe, Steven W.; Amano, Kagehiro**
PATENT ASSIGNEE(S): Regents of the University of Michigan, USA; Kaufman, Randal J.; Pipe, Steven W.; Amano, Kagehiro
SOURCE: PCT Int. Appl., 57 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9740145	A1	19971030	WO 1997-US6563	19970424
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, US, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9732027	A1	19971112	AU 1997-32027	19970424
EP 910628	A1	19990428	EP 1997-927596	19970424
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2000511407	T2	20000905	JP 1997-538216	19970424
US 2003148953	A1	20030807	US 2002-283648	20021029

PRIORITY APPLN. INFO.:

US 1996-16117P	P	19960424
US 1996-17785P	P	19960515
WO 1997-US6563	W	19970424
US 1997-980038	B1	19971126
US 2001-819098	A2	20010411
US 2002-122264	A2	20020411

AB Novel purified and isolated nucleic acid sequences encoding procoagulant-active FVIII proteins are described. To determine whether

specific amino acid sequences within FVIII A-domain inhibit secretion, chimeric proteins containing the A1 and A2-domains of FVIII or FV were studied. The nucleic acid sequences of encode amino acid sequences corresponding to known human FVIII sequences where **residue Phe309** is **mutated.** The nucleic acid sequences also encode human FVIII sequences where the APC cleavage sites, **Arg336** and Ile562, are **mutated.** The nucleic acid sequences of sequences corresponding to known human FVIII sequences where the B-domain is **deleted**, the von Willebrand factor binding site is **deleted**, a thrombin cleavage site is **mutated** and an amino acid sequence spacer is **inserted** between the A2- and A3-domains. These nucleotide encode **factor VIII** proteins capable of secretion at levels higher than typically obtained with wild-type **factor VIII.** Methods of producing the FVIII proteins and pharmaceutical compns. containing the nucleotide sequences or proteins as well as methods of treating patients suffering from hemophilia are also provided. A lower dosage of protein may be administered to the hemophiliac patient during FVIII replacement therapy. By utilizing the proteins described, the total exposure of protein to the patient is reduced, thereby lowering the likelihood of inhibitor formation.

L20 ANSWER 22 OF 44 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 97450923 MEDLINE
DOCUMENT NUMBER: 97450923 PubMed ID: 9305856
TITLE: Mutagenesis of a potential immunoglobulin-binding protein-binding site enhances secretion of coagulation **factor VIII.**
AUTHOR: Swaroop M; Moussalli M; **Pipe S W; Kaufman R J**
CORPORATE SOURCE: Howard Hughes Medical Institute, University of Michigan Medical Center, Ann Arbor, Michigan 48109, USA.
CONTRACT NUMBER: HL52173 (NHLBI)
HL53777 (NHLBI)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1997 Sep 26) 272 (39) 24121-4.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199710
ENTRY DATE: Entered STN: 19971105
Last Updated on STN: 19971105
Entered Medline: 19971023

AB Coagulation **factor VIII** (FVIII) and factor V are homologous glycoproteins that have a domain structure of A1-A2-B-A3-C1-C2. FVIII is a heterodimer of the heavy chain (domains A1-A2-B) and the light chain (domains A3-C1-C2) in a metal ion-dependent association between the A1- and A3-domains. Previous studies identified a 110-amino acid region within the FVIII A1-domain that inhibits its secretion and contains multiple short peptide sequences that have potential to bind immunoglobulin-binding protein (BiP). FVIII secretion requires high levels of intracellular ATP, consistent with an ATP-dependent release from BiP. Site-directed mutagenesis was used to elucidate the importance of the potential BiP-binding sites in FVIII secretion. **Mutation** of **Phe** at position **309** to Ser or Ala enhanced the secretion of functional FVIII and reduced its ATP dependence. The F309S FVIII had a specific activity, thrombin activation profile, and heat

inactivation properties similar to those of wild-type FVIII. However, F309S FVIII displayed increased sensitivity to EDTA-mediated **inactivation** that is known to occur through metal ion chelation-induced dissociation of the heavy and light chains of FVIII. The results support that Phe309 is important in high affinity heavy and light chain interaction, and this correlates with a high affinity BiP-binding site. Introduction of the F309S **mutation** into other secretion defective FVIII **mutants** rescued their secretion, demonstrating the ability of the this **mutation** to improve secretion of **mutant** FVIII proteins retained in the cell.

L20 ANSWER 23 OF 44 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 1998004487 MEDLINE
DOCUMENT NUMBER: 98004487 PubMed ID: 9342326
TITLE: Characterization of a genetically engineered **inactivation-resistant** coagulation factor VIIIa.
AUTHOR: **Pipe S W; Kaufman R J**
CORPORATE SOURCE: Department of Pediatrics, University of Michigan Medical Center, Ann Arbor, MI 48109, USA.
CONTRACT NUMBER: HL52173 (NHLBI)
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1997 Oct 28) 94 (22) 11851-6. Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199712
ENTRY DATE: Entered STN: 19980109
Last Updated on STN: 19980109
Entered Medline: 19971204

AB Individuals with hemophilia A require frequent infusion of preparations of coagulation **factor VIII**. The activity of **factor VIII** (FVIII) as a cofactor for factor IXa in the coagulation cascade is limited by its instability after activation by thrombin. Activation of FVIII occurs through proteolytic cleavage and generates an unstable FVIII heterotrimer that is subject to rapid dissociation of its subunits. In addition, further proteolytic cleavage by thrombin, factor Xa, factor IXa, and activated protein C can lead to **inactivation**. We have engineered and characterized a FVIII protein, IR8, that has enhanced in vitro stability of FVIII activity due to **resistance** to subunit dissociation and proteolytic **inactivation**. FVIII was genetically engineered by **deletion** of **residues** 794-1689 so that the A2 domain is covalently attached to the light chain. Missense **mutations** at thrombin and activated protein C **inactivation** cleavage sites provided **resistance** to proteolysis, resulting in a single-chain protein that has maximal activity after a single cleavage after **arginine**-372. The specific activity of partially purified protein produced in transfected COS-1 monkey cells was 5-fold higher than wild-type (WT) FVIII. Whereas WT FVIII was **inactivated** by thrombin after 10 min in vitro, IR8 still retained 38% of peak activity after 4 hr. Whereas binding of IR8 to von Willebrand factor (vWF) was reduced 10-fold compared with WT FVIII, in the presence of an anti-light chain antibody, ESH8, binding of IR8 to vWF increased 5-fold. These results demonstrate that **residues** 1690-2332 of FVIII are sufficient to support high-affinity vWF binding. Whereas ESH8 inhibited WT **factor VIII** activity, IR8 retained its activity in

the presence of ESH8. We propose that **resistance** to A2 subunit dissociation abrogates inhibition by the ESH8 antibody. The stable FVIIIa described here provides the opportunity to study the activated form of this critical coagulation factor and demonstrates that proteins can be improved by rationale design through genetic engineering technology.

L20 ANSWER 24 OF 44 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 97:884080 SCISEARCH
THE GENUINE ARTICLE: YG425
TITLE: Human and bovine activated protein C (APC) display differential cleavage and **inactivation** of human **factor VIII** at **residues** **ARG3361** and **ARG562**.
AUTHOR: **Amano K (Reprint)**; Yamanak K; Arai M; Fay P J; **Kaufman R J**; Fukutake K
CORPORATE SOURCE: TOKYO MED COLL, DEPT CLIN PATHOL, TOKYO 160, JAPAN; UNIV ROCHESTER, DEPT MED, ROCHESTER, NY; UNIV MICHIGAN, DEPT BIOL CHEM, ANN ARBOR, MI 48109; UNIV MICHIGAN, HOWARD HUGHES MED INST, ANN ARBOR, MI 48109
COUNTRY OF AUTHOR: JAPAN; USA
SOURCE: BLOOD, (15 NOV 1997) Vol. 90, No. 10, Part 2, Supp. [1], pp. 3116-3116.
Publisher: W B SAUNDERS CO, INDEPENDENCE SQUARE WEST CURTIS CENTER, STE 300, PHILADELPHIA, PA 19106-3399.
ISSN: 0006-4971.
DOCUMENT TYPE: Conference; Journal
FILE SEGMENT: LIFE; CLIN
LANGUAGE: English
REFERENCE COUNT: 0

L20 ANSWER 25 OF 44 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 97:693591 SCISEARCH
THE GENUINE ARTICLE: XV801
TITLE: The effect of **Arg**(306) -> Ala and **Arg**(506) -> Gln **substitutions** in the **inactivation** of recombinant human factor Va by activated protein C and protein S
AUTHOR: Egan J O; Kalafatis M; Mann K G (Reprint)
CORPORATE SOURCE: UNIV VERMONT, DEPT BIOCHEM, COLL MED, HLTH SCI COMPLEX, BURLINGTON, VT 05405 (Reprint); UNIV VERMONT, DEPT BIOCHEM, COLL MED, BURLINGTON, VT 05405
COUNTRY OF AUTHOR: USA
SOURCE: PROTEIN SCIENCE, (SEP 1997) Vol. 6, No. 9, pp. 2016-2027.
Publisher: CAMBRIDGE UNIV PRESS, 40 WEST 20TH STREET, NEW YORK, NY 10011-4211.
ISSN: 0961-8368.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 58
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Factor Va (Na) is **inactivated** by activated protein C (APC) by cleavage of the heavy chain at **Arg**(306), **Arg**(506), and **Arg**(679). Site-directed mutagenesis of human factor V cDNA was used to **substitute** **Arg**(306) --> Ala (rfVa(306A)) and **Arg**(506) --> Gln (rfVa(506Q)). Both the single and double **mutants** (rfVa(306A/506Q)) were constructed. The activation of these procofactors by alpha-thrombin and their **inactivation** by APC were assessed in coagulation assays using factor V-deficient plasma.

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All recombinant and wild-type proteins had similar initial cofactor activity and identical activation products (a factor Va molecule composed of light and heavy chains). **Inactivation** of factor Va purified from human plasma (fVa(PLASMA)) in HBS Ca2+ +0.5% BSA or in conditioned media by APC in the presence of phospholipid vesicles resulted in identical **inactivation** profiles and displayed identical cleavage patterns. Recombinant wild-type factor Va (rfVa(WT)) was **inactivated** by APC in the presence of phospholipid vesicles at an overall rate slower than fVa(PLASMA). The rfVa(306A) and rfVa(506Q) **mutants** were each **inactivated** at rates slower than rfVa(WT) and fVa(PLASMA). Following a 90-min incubation with APC, rfVa(306A) and rfVa(506Q) retain approximately 30-40% of the initial cofactor activity. The double **mutant**, rfVa(306A/506Q), was completely **resistant** to cleavage and **inactivation** by APC retaining 100% of the initial cofactor activity following a 90-min incubation in the presence of APC. Recombinant fVa(WT), rfVa(306A), rfVa(506Q), rfVa(306A/506Q) were also used to evaluate the effect of protein S on the individual cleavage sites of the cofactor by APC. The initial rates of rfVa(WT) and rfVa(306A) **inactivation** in the presence of protein S were unchanged, indicating cleavage at **Arg** (506) is not affected by protein S. The initial rate of rfVa(506Q) **inactivation** was increased, suggesting protein S slightly accelerates the cleavage at **Arg**(306). Overall, the data demonstrate high specificity with respect to cleavage sites for APC on factor Va and demonstrate that cleavages of the cofactor at both **Arg**(306) **Arg**(506) are required for efficient factor Va **inactivation**.

L20 ANSWER 26 OF 44 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1997:249690 BIOSIS
DOCUMENT NUMBER: PREV199799548893
TITLE: Factor V-Leiden and thrombophilia.
AUTHOR(S): Kalafatis, Michael; Mann, Kenneth G. [Reprint author]
CORPORATE SOURCE: Dep. Biochem., Given Build., Health Sci. Complex, Univ. Vt. Coll. Med., Burlington, VT 05405-0068, USA
SOURCE: Arteriosclerosis Thrombosis and Vascular Biology, (1997) Vol. 17, No. 4, pp. 620-627.
ISSN: 1079-5642.
DOCUMENT TYPE: Article
General Review; (Literature Review)
LANGUAGE: English
ENTRY DATE: Entered STN: 13 Jun 1997
Last Updated on STN: 13 Jun 1997

L20 ANSWER 27 OF 44 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 97:326385 SCISEARCH
THE GENUINE ARTICLE: WV168
TITLE: Search for **mutations** in the genes for coagulation factors V and VIII with a possible predisposition to activated protein C **resistance**
AUTHOR: Bokarewa M I (Reprint); Falk G; Bremme K; Blomback M; Wiman B
CORPORATE SOURCE: KAROLINSKA HOSP, DEPT CLIN CHEM BLOOD COAGULAT RES, CLIN CHEM BLDG, S-17176 STOCKHOLM, SWEDEN (Reprint); KAROLINSKA HOSP, DEPT WOMAN & CHILD HLTH, S-10401 STOCKHOLM, SWEDEN
COUNTRY OF AUTHOR: SWEDEN
SOURCE: EUROPEAN JOURNAL OF CLINICAL INVESTIGATION, (APR 1997) Vol. 27, No. 4, pp. 340-345.
Publisher: BLACKWELL SCIENCE LTD, OSNEY MEAD, OXFORD,

OXON, ENGLAND OX2 0EL.
ISSN: 0014-2972.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; CLIN
LANGUAGE: English
REFERENCE COUNT: 26

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A total of 74 non-pregnant women with a previous episode of thrombosis were investigated for activated protein C (APC) **resistance** in the aPTT-based and factor Ma-X-based assays and for the presence of **mutations** in all APC-cleavage sites in the heavy chains of factor V and **factor VIII**. DNA fragments were amplified with the polymerase chain reaction (PCR) and those encoding for the **Arg**-306 and **Arg**-506 (factor V) and for **Arg**-740 (**factor VIII**) cleavage sites were subjected to restriction enzyme analysis. DNA fragments of 29 selected patients corresponding to the **Arg**-306 and **Arg**-679 cleavage sites in factor V, and to the **Arg-336** and **Arg-562** cleavage sites in factor Vm were sequenced. APC **resistance** was found in 40 cases, using the aPTT-based assay and in 35, using the factor Ma-X-based assay (23 patients were APC **resistant** in both assays), whereas 22 individuals had a normal response to APC. Forty-three patients carried **Arg**-506 to Gin **mutation** in factor V. No other polymorphism or **mutation** was found in the genes for factors V or Vm in the vicinity of the APC cleavage sites. It was concluded that the difference in response to APC in the two assays may not be explained by the presence of **mutations** in the APC cleavage sites of factor V and factor Vm in this group of patients. The data do not exclude the presence of **mutations** elsewhere in the factor V or **factor VIII** genes.

L20 ANSWER 28 OF 44 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 97210670 EMBASE
DOCUMENT NUMBER: 1997210670
TITLE: Regulation of thrombin formation by activated protein C: Effect of the factor V Leiden **mutation.**
AUTHOR: Tans G.; Nicolaes G.A.F.; Rosing J.
CORPORATE SOURCE: Dr. J. Rosing, Department of Biochemistry, Cardiovascular Res. Inst. Maastricht, Maastricht University, P.O. Box 616, 6200 MD Maastricht, Netherlands
SOURCE: Seminars in Hematology, (1997) 34/3 (244-255).
Refs: 92
ISSN: 0037-1963 CODEN: SEHEA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 018 Cardiovascular Diseases and Cardiovascular Surgery
022 Human Genetics
025 Hematology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Activated Protein C (APC) **resistance**, one of the most common genetic risk factors for venous thrombosis, is caused by a single base **mutation** (G1691 → A) in the factor V (FV) gene resulting in the replacement of **Arg506** by Gln at a predominant cleavage site for APC. Great progress in understanding the mechanism of downregulation of FVa activity via the protein C pathway has been achieved by studying APC-mediated **inactivation** of FVa purified from homozygous APC-**resistant** individuals. This review briefly summarizes the role of

FVa in prothrombin activation and the structure- function relationship of FV and FVa. Subsequently, APC-dependent **inactivation** of FVa and FVa Leiden and its modulation by protein S and factor Xa in model systems containing purified proteins in discussed. FV also has a function in increasing the **inactivation** of FVIII/VIIIa by APC. This cofactor activity appears diminished in FV Leiden. Thus, an intricate mechanism of regulation of thrombin formation via the protein C pathway is starting to emerge. Extensive studies in plasma milieu will be needed to gain more insight into the relation between the presence of FV Leiden and impaired downregulation of thrombin formation in APC-**resistant** individuals.

L20 ANSWER 29 OF 44 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1998:60942 BIOSIS
DOCUMENT NUMBER: PREV199800060942
TITLE: Human and bovine activated protein C (APC) display differential cleavage and **inactivation** of human **factor VIII** at **residues** **ARG3361** and **ARG562**.
AUTHOR(S): **Amano, K.** [Reprint author]; Yamana, K.; Arai, M.; Fay, P. J.; **Kaufman, R. J.**; Fukutake, K.
CORPORATE SOURCE: Dep. Clin. Pathol., Tokyo Med. Coll., Tokyo, Japan
SOURCE: Blood, (Nov. 15, 1997) Vol. 90, No. 10 SUPPL. 1 PART 2, pp. 90B. print.
Meeting Info.: Thirty-ninth Annual Meeting of the American Society of Hematology. San Diego, California, USA. December 5-9, 1997. The American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 30 Jan 1998
Last Updated on STN: 30 Jan 1998

L20 ANSWER 30 OF 44 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 96219649 MEDLINE
DOCUMENT NUMBER: 96219649 PubMed ID: 8639840
TITLE: Comparison of activated protein C/protein S-mediated **inactivation** of human **factor VIII** and factor V.
AUTHOR: Lu D; Kalafatis M; Mann K G; Long G L
CORPORATE SOURCE: Department of Biochemistry, College of Medicine, University of Vermont, Burlington, VT 05405, USA.
CONTRACT NUMBER: HL34575 (NHLBI)
HL39745 (NHLBI)
HL46703 (NHLBI)
+
SOURCE: BLOOD, (1996 Jun 1) 87 (11) 4708-17.
Journal code: 7603509. ISSN: 0006-4971.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199607
ENTRY DATE: Entered STN: 19960726
Last Updated on STN: 19970203
Entered Medline: 19960718
AB The proteolytic cleavage and subsequent **inactivation** of recombinant human **factor VIII** (rhFVIII) and human

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factor VIIIa (rhFVIIIa) by recombinant human activated protein C (rAPC) was analyzed in the presence and absence of human protein S and human factor V (FV). Membrane-bound rhFVIIIa spontaneously looses most of its initial cofactor activity after 15 minutes of incubation at pH 7.4. The remaining activity can be eliminated after incubation with rAPC. Complete **inactivation** of the membrane-bound rhFVIII and rhFVIIIa by APC correlates with cleavage at **Arg336**. The **inactivation** of rhFVIII and human plasma FV by rAPC were also compared. Under similar experimental conditions, complete **inactivation** of membrane-bound FVIII (60 nmol/L) by rAPC (10 nmol/L) requires 4 hours of incubation, in contrast to 5 minutes for FV (60 nmol/L). The presence of protein S (100 nmol/L) enhances rhFVIII **inactivation** by rAPC by 6.4-fold and FVa **inactivation** by twofold, whereas membrane-bound FV showed no protein S dependence during **inactivation**. The addition of human FV to the APC/protein S **inactivation** mixture increases by approximately twofold the rate of **inactivation** of rhFVIII. The effect of FV on the rhFVIII **inactivation** by APC is protein S-dependent, because FV alone has no effect on the **inactivation** rate of rhFVIII by APC. Western blotting using a monoclonal antibody that recognizes an epitope between amino acid **residues** 307 and 506 of human FV showed that FV was completely cleaved by APC at the beginning of the rhFVIII **inactivation** process. These data suggest that FV fragments derived from the B region of the procofactor after incubation of the membrane-bound procofactor with APC, but not intact single-chain FV, stimulate APC activity in the presence of protein S. rhFVIII, FV, and rhFVIIIa were not **inactivated** by Glu20-->Ala-**substituted** rAPC (rAPCgamma20A), and membrane-bound factor Va was only partially **inactivated**. Our data suggest that (1) FV and FVa are the physiologically significant substrates for APC **inactivation** and (2) membranes-bound APC-treated FV is a cofactor for the APC **inactivation** of rhFVIII only in the presence of the intact form of protein S.

L20 ANSWER 31 OF 44 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 9
ACCESSION NUMBER: 1996:130507 HCAPLUS
DOCUMENT NUMBER: 124:169161
TITLE: Activated protein C-catalyzed proteolysis of factor VIIIa alters its interactions within factor Xase
AUTHOR(S): Regan, Lisa M.; O'Brien, Lynn M.; Beattie, Tammy L.; Sudhakar, Katakam; Walker, Frederick J.; Fay, Philip J.
CORPORATE SOURCE: Dep. Biochem. Med., Univ. Rochester Sch. Med., Rochester, NY, 14642, USA
SOURCE: Journal of Biological Chemistry (1996), 271(8), 3982-7
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Factor VIIIa, the cofactor for the factor IXa-dependent conversion of factor X to factor Xa, is proteolytically **inactivated** by activated protein C (APC). APC cleaves at two sites in factor VIIIa, **Arg336**, near the C terminus of the A1 subunit; and **Arg562**, bisecting the A2 subunit (Fay, P., Smudzin, T., and Walker, F. (1991) J. Biol. Chemical 266, 20139-20145). Factor VIIIa increased the fluorescence anisotropy of fluorescein-**Phe-Phe-Arg** factor IXa (Fl-FFR-FIXa; Kd = 42.4 nM), whereas cleavage of factor VIIIa by APC eliminated this property. Isolation of the APC-cleaved A1/A3-C1-C2 dimer (A1336/A3-C1-C2), and the fragments derived from cleaved A2 subunit

(A2N/A2C), permitted dissection of the roles of individual cleavages in cofactor **inactivation.** Intact A1/A3-C1-C2 dimer increased F1-FFR-FIXa anisotropy and bound factor X in a solid phase assay, while these activities were absent in the a1336/A3-C1-C2. However, the **residues** removed by this cleavage, Met337-**Arg372**, did not directly participate in these functions since neither a synthetic peptide to this sequence nor an anti-peptide polyclonal antibody blocked these activities using intact dimer. CD spectral anal. of the intact and truncated dimers indicated reduced α and/or β content in the latter. The A1/A3-C1-C2 dimer plus A2 subunit reconstitutes cofactor activity and produced a factor VIIIa-like effect on the anisotropy of F1-FFR-FIXa. However, when A2 was replaced by the A2N/A2C fragments, the resulting fluorescence signal was equivalent to that observed with the dimer alone. These results indicate that APC **inactivates** the cofactor at two levels within the intrinsic factor Xase complex. Cleavage of either subunit modulates the factor IXa active site, suggesting an essential synergy of interactive sites in factor VIIIa. Furthermore, cleavage of the A1 site alters the conformation of a factor X binding site within that subunit, thereby reducing the affinity of cofactor for substrate.

L20 ANSWER 32 OF 44 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 96:193581 SCISEARCH
THE GENUINE ARTICLE: TY265
TITLE: ABSENCE OF **MUTATIONS** AT THE ACTIVATED PROTEIN-C CLEAVAGE SITES OF **FACTOR-VIII** IN 125 PATIENTS WITH VENOUS THROMBOSIS
AUTHOR: ROELSE J C; KOOPMAN M M W; BULLER H R; TENCATE J W; MONTARULI B; VANMOURIK J A; VOORBERG J (Reprint)
CORPORATE SOURCE: NETHERLANDS RED CROSS, BLOOD TRANSFUS SERV, CENT LAB, DEPT BLOOD COAGULAT, PLESMANLAAN 125, 1066 CX AMSTERDAM, NETHERLANDS (Reprint); NETHERLANDS RED CROSS, BLOOD TRANSFUS SERV, CENT LAB, DEPT BLOOD COAGULAT, 1066 CX AMSTERDAM, NETHERLANDS; UNIV AMSTERDAM, ACAD MED CTR, CTR THROMBOSIS HAEMOSTASIS & ATHEROSCLEROSIS INFL, 1105 AZ AMSTERDAM, NETHERLANDS
COUNTRY OF AUTHOR: NETHERLANDS
SOURCE: BRITISH JOURNAL OF HAEMATOLOGY, (MAR 1996) Vol. 92, No. 3, pp. 740-743.
ISSN: 0007-1048.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; CLIN
LANGUAGE: ENGLISH
REFERENCE COUNT: 12
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB **Resistance** to activated protein C (APC), caused by a **mutation** at amino acid position **Arg**(506) of the factor V gene, has recently been identified as the most prevalent genetic defect associated with venous thrombosis. Similarly to factor V, **mutations** at the cleavage sites of **factor VIII** for APC may occur in patients with venous thrombosis. Here we have analysed 125 consecutive patients with incidental or recurrent venous thromboembolism for the presence of **mutations** at the cleavage sites for APC at amino acid positions **Arg**(**336**) and **Arg**(**562**) of **factor VIII**. Our findings indicate that **mutations** at these amino acid positions of **factor VIII** do not occur in the patient group analysed.

Search completed by David Schreiber 308-4292

L20 ANSWER 33 OF 44 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 96:18157 SCISEARCH
THE GENUINE ARTICLE: TH910
TITLE: COMPLETE APC-MEDIATED **INACTIVATION** OF **FACTOR-VIII** REQUIRES CLEAVAGE AT BOTH **ARG336** AND **ARG562** - IMPLICATIONS FOR THE APC **RESISTANCE** TEST
AUTHOR: **AMANO K (Reprint)**; MOUSALLI M; MICHNICK D A; **KAUFMAN R J**
CORPORATE SOURCE: UNIV MICHIGAN, DEPT BIOL CHEM, ANN ARBOR, MI, 48109; UNIV MICHIGAN, HOWARD HUGHES MED INST, ANN ARBOR, MI, 48109; GENET INST INC, CAMBRIDGE, MA, 02140
COUNTRY OF AUTHOR: USA
SOURCE: BLOOD, (15 NOV 1995) Vol. 86, No. 10, Supp. 1, pp. 1776. ISSN: 0006-4971.
DOCUMENT TYPE: Conference; Journal
FILE SEGMENT: LIFE; CLIN
LANGUAGE: ENGLISH
REFERENCE COUNT: No References

L20 ANSWER 34 OF 44 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 95:265481 SCISEARCH
THE GENUINE ARTICLE: QR243
TITLE: PROPOSED CAUSE OF MARKED VASOPRESSIN **RESISTANCE** IN A FEMALE WITH AN X-LINKED RECESSIVE V2 RECEPTOR ABNORMALITY
AUTHOR: MOSES A M (Reprint); SANGANI G; MILLER J L
CORPORATE SOURCE: SUNY HLTH SCI CTR, DEPT MED, 750 E ADAMS ST, SYRACUSE, NY, 13210 (Reprint); SUNY HLTH SCI CTR, DEPT PATHOL, SYRACUSE, NY, 13210
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF CLINICAL ENDOCRINOLOGY AND METABOLISM, (APR 1995) Vol. 80, No. 4, pp. 1184-1186. ISSN: 0021-972X.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; CLIN
LANGUAGE: ENGLISH
REFERENCE COUNT: 29

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Almost all cases of congenital nephrogenic diabetes insipidus (NDI) are transmitted in an X-linked recessive manner by an asymptomatic carrier female to her affected son. Severe symptomatic NDI has not previously been reported in a female with X-linked recessive NDI. Each of the three members of this family has an abnormal V-2 receptor gene, which results in truncation of the V-2 receptor beginning at **arginine**-337. This prematurely terminates the receptor at the carboxy-terminal tail and very likely disrupts receptor function. The son has an abnormal V-2 receptor gene on his X-chromosome, whereas the mother and daughter have one normal and one abnormal gene for the V-2 receptor. The infusion of desmopressin into the mother and son reveals a total lack of antidiuretic response, whereas the daughter increases urinary osmolality normally. The plasma **factor VIII** concentration after the infusion of desmopressin in the son does not rise, whereas the mother and daughter have half of the normal **factor VIII** response, similar to asymptomatic female carriers of NDI. These responses to desmopressin in daughter and son are those of a typical carrier female and male affected with NDI. In contrast, the mother acts as an NDI patient when the urine concentration is measured, but acts as a carrier in terms of the **factor VIII** response to desmopressin. We postulate that

the renal tubular cells of the mother demonstrate extreme lyonization of X-chromosome **inactivation**, whereas in the tissue that subserves the hematological response to desmopressin, X-chromosome **inactivation** followed a more typically random distribution.

L20 ANSWER 35 OF 44 MEDLINE on STN DUPLICATE 10
ACCESSION NUMBER: 95399286 MEDLINE
DOCUMENT NUMBER: 95399286 PubMed ID: 7669667
TITLE: A chromogenic assay for activated protein C **resistance.**
AUTHOR: Varadi K; Moritz B; Lang H; Bauer K; Preston E; Peake I; Rivard G E; Keil B; Schwarz H P
CORPORATE SOURCE: Research Laboratories of Immuno AG, Vienna, Austria.
SOURCE: BRITISH JOURNAL OF HAEMATOLOGY, (1995 Aug) 90 (4) 884-91. Journal code: 0372544. ISSN: 0007-1048.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199510
ENTRY DATE: Entered STN: 19951026
Last Updated on STN: 19951026
Entered Medline: 19951018

AB **Resistance** to activated protein C (APC) diagnosed on the basis of prolongation of clotting time in an activated partial thromboplastin time (aPTT) assay is now considered a major cause of inherited thrombophilia. The majority of patients with APC **resistance** carry a factor V molecule with a point **mutation** at one APC cleavage site (**Arg506Gln**) which prevents the optimal **inactivation** of activated factor V by APC. To overcome the limitations of aPTT-based assays in the diagnosis of APC **resistance**, we have developed a chromogenic assay which is based on the capacity of APC to limit the generation of factor Xa by **inactivating** factor VIIIa in plasma. The ratio of the factor Xa amidolytic activity in a sample without APC to its factor Xa activity with the addition of APC reflects the response of the plasma coagulation system to APC. The normal range in 44 healthy individuals was 1.62-2.06. APC response ratios as measured by the chromogenic assay correlated with ratios measured by the aPTT assay and were below the normal range in 23/24 individuals with **Arg506Gln mutant** factor V from three different families with familial thrombosis and from 11 unrelated asymptomatic individuals. In reconstitution experiments, purified factor V corrected the decreased APC response in plasma samples from patients with the **Arg506Gln mutation** as well as with factor V deficiency, and increased the APC response in normal plasma, whereas the addition of activated factor V had no enhancing effect.

L20 ANSWER 36 OF 44 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1996:49473 BIOSIS
DOCUMENT NUMBER: PREV199698621608
TITLE: Complete APC-mediated **inactivation** of **factor VIII** requires cleavage at both **Arg336** and **Arg562**: Implications for the APC **resistance** test.
AUTHOR(S): **Amano, K.** [Reprint author]; Moussalli, M. [Reprint author]; Michnick, D. A. [Reprint author]; **Kaufman, R. J.**
CORPORATE SOURCE: Dep. Biol. Chem., Howard Hughes Med. Inst., Univ. Michigan, Ann Arbor, MI, USA

Search completed by David Schreiber 308-4292

SOURCE: Blood, (1995) Vol. 86, No. 10 SUPPL. 1, pp. 447A.
Meeting Info.: 37th Annual Meeting of the American Society of Hematology. Seattle, Washington, USA. December 1-5, 1995.
CODEN: BLOOAW. ISSN: 0006-4971.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 2 Feb 1996
Last Updated on STN: 13 Mar 1996

L20 ANSWER 37 OF 44 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 95:555804 SCISEARCH
THE GENUINE ARTICLE: RN048
TITLE: **RESISTANCE** TO ACTIVATED PROTEIN-C DUE TO **MUTATED** FACTOR-V AS A NOVEL CAUSE OF INHERITED THROMBOPHILIA
AUTHOR: DESTEFANO V (Reprint); LEONE G
CORPORATE SOURCE: CATHOLIC UNIV SACRED HEART, IST SEMEIOT MED, DIV EMATOL, LARGO GEMELLI 8, I-00168 ROME, ITALY (Reprint)
COUNTRY OF AUTHOR: ITALY
SOURCE: HAEMATOLOGICA, (JUL/AUG 1995) Vol. 80, No. 4, pp. 344-356.
ISSN: 0390-6078.
DOCUMENT TYPE: General Review; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 99
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Inherited **resistance** to activated protein C (APC) was recently recognized as a novel cause underlying venous thrombophilia. In most cases APC-**resistance** is due to a single point **mutation** in the factor V gene leading to a replacement of **Arg506** with Gin (factor V Leiden). Amino add **substitution** occurs at one of the APC cleavage sites of factor Va, rendering it **resistant** to APC **inactivation.** Plasma anticoagulant response to exogenous APC as a simple diagnostic assay of APC **resistance** shows good sensitivity and specificity as compared to gene analysis, yet standardization of the results needs to be improved. The APC-**resistance** trait is present in 2%-6% of the general population and was found to be associated with venous thrombophilia in about 20% of patients with unexplained thrombosis. Clinical features are substantially similar to other congenital plasma abnormalities predisposing to thrombosis (antithrombin III, protein C, protein S deficiencies); yet the overall clinical penetrance of the defect seems lower, at least for the heterozygous condition. Preliminary data suggest a higher risk of thrombosis in APC-**resistant** homozygous individuals or in patients exhibiting APC-**resistance** together with other thrombophilic genetic defects. To date, genetically determined APC-**resistance** does not seem to play a significant role in the development of arterial thrombotic disease.

L20 ANSWER 38 OF 44 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 94:493844 SCISEARCH
THE GENUINE ARTICLE: PB317
TITLE: FACTOR-VIIIA A2-SUBUNIT **RESIDUES**-558-565 REPRESENT A FACTOR-IXA INTERACTIVE SITE
AUTHOR: FAY P J (Reprint); BEATTIE T; HUGGINS C F; REGAN L M
CORPORATE SOURCE: UNIV ROCHESTER, MED CTR, SCH MED & DENT, DEPT MED, HEMATOL UNIT, 601 ELMWOOD AVE, ROCHESTER, NY, 14642 (Reprint);

UNIV ROCHESTER, SCH MED & DENT, DEPT BIOCHEM, ROCHESTER, NY, 14642
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (12 AUG 1994) Vol. 269, No. 32, pp. 20522-20527.
ISSN: 0021-9258.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 34

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Factor VIIIa is a non-covalent heterotrimer of A1, A2, and A3-C1-C2 subunits. Previously, we speculated that the central portion of the A2 subunit, in and around the activated protein C-sensitive bond at **Arg(562)**-Gly (Fay, P. J., Smudzin, T. M., and Walker, F J. (1991) J. Biol. Chemical 266, 20139-20145), is important for macromolecular interactions within the factor Xase enzyme complex. A peptide corresponding to **factor VIII residues** 558-565, SVDQRGNQ and designated FVIII558-565, was chemically synthesized and inhibited factor Xa generation in a purified system with an apparent K-I of 105 mu M. Tryptic cleavage of FVIII558-565 eliminated its inhibitory activity, whereas a scrambled sequence version of the peptide possessed <30% the inhibitory activity of the native version. Overlapping peptides FVIII556-564 and FVIII561-569 were also inhibitory and confirmed the importance of **residues** in and around the scissile bond for functional factor Xase. Kinetic analysis revealed that peptide-mediated inhibition was non-competitive with respect to factor X. However, increasing factor IXa concentration overcame the observed inhibition. Furthermore, the peptide inhibited the factor IXa-dependent enhancement of factor VIIIa reconstituted from isolated A1/A3-C1-C2 dimer plus A2 subunit. Isolated **factor VIII** heavy chain (contiguous A1-A2 domains) was cleaved at **Arg(336)** by an equimolar concentration of factor IXa in a reaction that was phospholipid-independent. No proteolysis of the isolated A1 subunit was observed in a similar reaction. These results indicate that the A2 subunit sequence delineated by **residues** 558-565 contributes to the interaction of cofactor with protease and that this interaction is essential for intrinsic factor Xase activity. Furthermore, that this peptide blocks both factor Xase activity and the capacity of factor IXa to stabilize the labile factor VIIIa heterotrimer suggest that this latter property is of physiologic significance.

ACCESSION NUMBER: 94202583 EMBASE
DOCUMENT NUMBER: 1994202583
TITLE: Factor IXa protects factor VIIIa from activated protein C. Factor IXa inhibits activated protein C-catalyzed cleavage of factor VIIIa at **ARG562**.
AUTHOR: Regan L.M.; Lamphear B.J.; Huggins C.F.; Walker F.J.; Fay P.J.
CORPORATE SOURCE: Hematology Unit, Rochester University Medical Center, 601 Elmwood Ave.,Rochester, NY 14642, United States
SOURCE: Journal of Biological Chemistry, (1994) 269/13 (9445-9452).
ISSN: 0021-9258 CODEN: JBCHA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 025 Hematology
029 Clinical Biochemistry

LANGUAGE: English
SUMMARY LANGUAGE: English
AB Factor VIIIa is **inactivated** by both factor IXa and activated protein C. The latter protease rapidly attacked a site at **Arg562** (A2 subunit), whereas both proteases slowly cleaved factor VIIIa at **Arg336** (A1 subunit). Cofactor **inactivation** catalyzed by activated protein C was 8-fold faster than that catalyzed by factor IXa. Simultaneous reaction of factor VIIIa with the two enzymes resulted in a rate of **inactivation** intermediate to that observed for the individual proteases. Under these conditions, the activated protein C-catalyzed cleavage at **Arg562** was inhibited such that cofactor **inactivation** resulted primarily from cleavage at **Arg336**. **Substitution** of factor IXa modified in its active site with 6-(dimethylamino)-2- naphthalenesulfonyl-glutamylglycylarginyl chloromethyl ketone (DEGR-IXa) for the native enzyme yielded a similar rate of activated protein C-catalyzed cleavage at the A1 site, whereas cleavage at the A2 site was virtually eliminated. However, the inclusion of protein S resulted in a marked increase in cleavage at the A2 site that correlated with an increased rate of cofactor **inactivation.** Active site-modified activated protein C inhibited the factor IXa-dependent enhancement of factor VIIIa reconstitution from isolated subunits. In addition, the factor VIIIa-dependent fluorescence enhancement of DEGR-activated protein C was inhibited by EGR-IXa. These results indicate that factor IXa can reduce the rate of activated protein C-catalyzed cofactor **inactivation** by selectively blocking cleavage at the A2 domainal site, an effect reversed by protein S. One mechanism consistent with the reciprocal inhibitory effects of the proteases is that activated protein C and factor IXa occupy overlapping sites on the cofactor. Thus, factor IXa may protect factor VIIIa by preventing activated protein C binding.

L20 ANSWER 40 OF 44 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 94:733187 SCISEARCH
THE GENUINE ARTICLE: PR345
TITLE: CONSTRUCTION AND CHARACTERIZATION OF THROMBIN-**RESISTANT** VARIANTS OF RECOMBINANT HUMAN PROTEIN-S
AUTHOR: CHANG G T G; AALDERING L; HACKENG T M; REITSMA P H; BERTINA R M; BOUMA B N (Reprint)
CORPORATE SOURCE: UNIV UTRECHT HOSP, DEPT HAEMATOL, HEIDELBERGLAAN 100, POB 85500, 3508 GA UTRECHT, NETHERLANDS (Reprint); UNIV UTRECHT HOSP, DEPT HAEMATOL, 3508 GA UTRECHT, NETHERLANDS; UNIV LEIDEN HOSP, DEPT HAEMATOL, THROMBOSIS & HAEMOSTASIS RES CTR, 2300 RC LEIDEN, NETHERLANDS
COUNTRY OF AUTHOR: NETHERLANDS
SOURCE: THROMBOSIS AND HAEMOSTASIS, (NOV 1994) Vol. 72, No. 5, pp. 693-697.
ISSN: 0340-6245.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 28
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Protein S is a vitamin K-dependent plasma protein that functions as a cofactor of activated protein C (APC) in the **inactivation** of coagulation factors Va and VIIIa.

Protein S, migrates as a doublet on reduced SDS polyacrylamide gel electrophoresis. This heterogeneity in molecular weight has been explained by limited proteolysis of protein S. Human protein S contains at **Arg**-49, **Arg**-60 and **Arg**-70 three potential

Search completed by David Schreiber 308-4292

cleavage sites. Whether cleavage occurs at all three sites is not known. To study the role of these **arginine residues** in human protein S, we have replaced them by leucine or isoleucine. All seven possible variants were constructed: three variants with single **mutations** (R49L, R60L, R70I), three variants with double **mutations** (R49L/R60L, R60L/R70I, R49L/R70I) and one variant with a triple **mutation** (R49L/R60L/R70I). On reduced SDS polyacrylamide gels the single and double variants migrate as a doublet just like the wild type protein S. The triple variant migrates as a single band at a molecular weight corresponding to the upper band of the doublet. The upper band of the single and double variants but not of the triple variant could be converted into the lower band by thrombin treatment.

All variants showed cofactor activity to APC in a clotting assay. After thrombin treatment, this cofactor activity was abolished for the single (R49L, R60L, R70I) and double variants (R49L/R60L, R60L/R70I, R49L/R70I), while the triple variant (R49L/R60L/R70I) tested at several concentrations, retained its cofactor activity completely, suggesting **resistance** to thrombin. This shows that thrombin can cleave at all three **arginine** sites and that cleavage at each of these sites results in the loss of APC cofactor activity. Finally, all variants bind to C4b-binding protein with an affinity similar as the wild type recombinant molecule.

L20 ANSWER 41 OF 44 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 94:254154 SCISEARCH
THE GENUINE ARTICLE: NJ860
TITLE: **MUTATION** IN BLOOD-COAGULATION FACTOR-V ASSOCIATED WITH **RESISTANCE** TO ACTIVATED PROTEIN-C
AUTHOR: BERTINA R M (Reprint); KOELEMAN B P C; KOSTER T; ROSENDAAL F R; DIRVEN R J; DERONDE H; VANDERVELDEN P A; REITSMA P H
CORPORATE SOURCE: UNIV LEIDEN HOSP, CTR HEMOSTASIS & THROMBOSIS RES, BLDG 1-C2R, POB 9600, 2300 RC LEIDEN, NETHERLANDS (Reprint); UNIV LEIDEN HOSP, DEPT CLIN EPIDEMIOL, 2300 RC LEIDEN, NETHERLANDS
COUNTRY OF AUTHOR: NETHERLANDS
SOURCE: NATURE, (05 MAY 1994) Vol. 369, No. 6475, pp. 64-67. ISSN: 0028-0836.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: PHYS; LIFE; AGRI
LANGUAGE: ENGLISH
REFERENCE COUNT: 30

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB ACTIVATED protein C (APC) is a serine protease with potent anticoagulant properties, which is formed in blood on the endothelium from an inactive precursor(1). During normal haemostasis, APC limits clot formation by proteolytic **inactivation** of factors Va and VIIIa (reference 2). To do this efficiently the enzyme needs a nonenzymatic cofactor, protein S (reference 3). Recently it was found that the anticoagulant response to APC (APC **resistance**)(4) was very weak in the plasma of 21% of unselected consecutive patients with thrombosis(5) and about 50% of selected patients with a personal or family history of thrombosis(6,7); moreover, 5% of healthy individuals show APC **resistance**, which is associated with a sevenfold increase in the risk for deep vein thrombosis(5). Here we demonstrate that the **phenotype** of APC **resistance** is associated with heterozygosity or homozygosity for a single point **mutation** in the factor V gene (at nucleotide position 1,691, G --> A **substitution**) which predicts the synthesis of a factor V molecule (FV Q506, or FV Leiden) that is not

properly **inactivated** by APC. The allelic frequency of the **mutation** in the Dutch population is similar to 2% and is at least tenfold higher than that of all other known genetic risk factors for thrombosis (protein C (reference 8), protein S (reference 9), antithrombin(10) deficiency) together.

L20 ANSWER 42 OF 44 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 93:25579 SCISEARCH
THE GENUINE ARTICLE: KF241
TITLE: RECOMBINANT **FACTOR-VIII** (ANTIHEMOPHILIC-A) - STRUCTURE-FUNCTION RELATIONSHIP
AUTHOR: BIHOREAU N (Reprint)
CORPORATE SOURCE: TM INNOVAT, DEPT RECH & DEV BIOTECHNOL, 3 AVE TROP, F-91943 LES ULIS, FRANCE (Reprint)
COUNTRY OF AUTHOR: FRANCE
SOURCE: M S-MEDECINE SCIENCES, (DEC 1992) Vol. 8, No. 10, pp. 1043-1050.
ISSN: 0767-0974.
DOCUMENT TYPE: General Review; Journal
FILE SEGMENT: LIFE
LANGUAGE: French
REFERENCE COUNT: No References Keyed

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB **Factor VIII** is a glycoprotein which acts as a cofactor in the blood coagulation pathway. Sequence modifications or a deficiency in **factor VIII** cause a serious bleeding disorder called haemophilia A. Gene cloning has greatly contributed to the understanding of the **factor VIII** structure and function. The primary sequence, deduced from the human cDNA, predicts a mature polypeptide of 2332 aminoacids which exhibits three domains arranged as follows : A1-A2-B-A3-C1-C2. **Factor VIII** is present in plasma as a complex corresponding to the association, by a divalent ion, of a 210 kDa heavy chain (A1-A2-B) to a 80 kDa light chain (A3-C1-C2). Cleavages of the B domain lead to different heterodimers of Mr ranging from 210-80 to 90-80 kDa. **Factor VIII** acts in the blood coagulation pathway in its activated or **inactivated** forms after proteolysis by thrombin or activated protein C, respectively. Site-directed mutagenesis has allowed an analysis of the structural requirements for **factor VIII** function. Thrombin activation corresponds to proteolysis on the heavy and light chains after the **Arg** 372 and 1689, respectively, to generate the activated trimer A1/A2/A3-C1-C2. Dissociation of the A2 domain from this complex leads to **factor VIII inactivation**. By contrast, **inactivation** with the activated protein C corresponds to the proteolysis of the heavy chain after **Arg336** to generate the A1/A3-C1-C2 dimer. Considering these structural requirements and that the B region is dispensable for procoagulant activity, different variants of **factor VIII** comprising partial or complete **deletions** of the B domain have been constructed. These variants, which are better secreted than the complete protein, are active to restore coagulation in vitro and in vivo in hemophilic dogs. The clinical investigations have shown that the complete recombinant **factor VIII** is efficacious for treatment of haemophilia A.

L20 ANSWER 43 OF 44 MEDLINE on STN DUPLICATE 12
ACCESSION NUMBER: 88190085 MEDLINE
DOCUMENT NUMBER: 88190085 PubMed ID: 3128786
TITLE: Proteolytic requirements for thrombin activation of anti-hemophilic factor (**factor VIII**).

Search completed by David Schreiber 308-4292

Desai 09/819,098

AUTHOR: Pittman D D; **Kaufman R J**
CORPORATE SOURCE: Genetics Institute, Cambridge, MA 02140.
CONTRACT NUMBER: 2R44HL35946-02 (NHLBI)
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1988 Apr) 85 (8) 2429-33.
Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198805
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 19970203
Entered Medline: 19880520

AB **Factor VIII** functions in the intrinsic pathway of coagulation as the cofactor for factor IXa proteolytic activation of factor X. Proteolytic cleavage is required for activation and may be responsible for **inactivation** of cofactor activity. To identify which of the multiple cleavages are required for activation and **inactivation** of **factor VIII**, site-directed DNA-mediated mutagenesis of the **factor VIII** cDNA was performed and the altered forms of **factor VIII** were expressed in COS-1 monkey cells and characterized. Conversion of **arginine residues** to isoleucine **residues** at the aminoterminal side of the cleavage sites at positions 740, 1648, and 1721 resulted in cleavage **resistance** at the modified site with no alteration in the in vitro procoagulant activity and the susceptibility to thrombin activation. Similar modification of the thrombin cleavage sites at either position 372 or position 1689 resulted in molecules with residual **factor VIII** activity but **resistant** to thrombin cleavage at the modified site and not susceptible to thrombin activation. Modification of the **arginine** to either an isoleucine or a lysine at **residue 336**, the site postulated for proteolytic **inactivation** by activated protein C, resulted in a **factor VIII** molecule with increased procoagulant activity. This increased activity may result from greater **resistance** to proteolytic **inactivation**. A model for the activation and **inactivation** of **factor VIII** is proposed.

L20 ANSWER 44 OF 44 MEDLINE on STN DUPLICATE 13
ACCESSION NUMBER: 88327107 MEDLINE
DOCUMENT NUMBER: 88327107 PubMed ID: 3137981
TITLE: **Mutations** of **factor VIII** cleavage sites in hemophilia A.
AUTHOR: Gitschier J; Kogan S; Levinson B; Tuddenham E G
CORPORATE SOURCE: Howard Hughes Medical Institute, University of California, San Francisco 94143.
SOURCE: BLOOD, (1988 Sep) 72 (3) 1022-8.
Journal code: 7603509. ISSN: 0006-4971.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 198810
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 19990129
Entered Medline: 19881019

AB Hemophilia A is caused by a defect in coagulation **factor**

Search completed by David Schreiber 308-4292

VIII, a protein that undergoes extensive proteolysis during its activation and **inactivation**. To determine whether some cases of hemophilia are caused by **mutations** in important cleavage sites, we screened patient DNA samples for **mutations** in these sites by a two-step process. Regions of interest were amplified from genomic DNA by repeated rounds of primer-directed DNA synthesis. The amplified DNAs were then screened for **mutations** by discriminant hybridization using oligonucleotide probes. Two cleavage site **mutations** were found in a survey of 215 patients. A nonsense **mutation** in the activated protein C cleavage site at amino acid **336** was discovered in a patient with severe hemophilia. In another severely affected patient, a mis-sense **mutation** results in a **substitution** of cysteine for **arginine** in the thrombin activation site at amino acid 1689. This defect is associated with no detectable **factor VIII** activity, but with normal levels of **factor VIII** antigen. The severe hemophilia in this patient was sporadic; analysis of the mother suggested that the **mutation** originated in her gametes or during her embryogenesis. The results demonstrate that this approach can be used to identify **factor VIII** gene **mutations** in regions of the molecule known to be important for function.